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11th International Coral Reef Symposium Proceedings

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US CORAL REEF TASK FORCE

Commitment to Coral Reef Conservation:

- Leadership
- Research
- Management
- Education

The U.S. Coral reef Task Force's (USCRTF) mission is to lead, coordinate, and strengthen U.S. government actions to better preserve and protect coral reef ecosystems. The Task Force is co-chaired by the Departments of Commerce and of the Interior, and includes leaders of 12 federal agencies, seven U.S. states and territories, and three freely associated states. To implement the policies and requirements of the Executive Order, the Task Force has provided a forum for coordinated planning and action among federal agencies, state and territorial governments, and nongovernmental partners. To fulfil its mission, the Task Force developed national strategies, targeted initiatives, and new partnerships to strengthen stewardship of the coral reef ecosystems in the United States territories, commonwealths, and internationally.

STATE OF FLORIDA

With the longest coastline of the 48 contiguous states, 41 aquatic preserves and three of the nation's National Estuarine Research Reserves, Florida is undeniably an ocean state. Over the last five years, Florida has established the benchmark for ocean protection by significantly restricting offshore drilling, establishing the Tortugas Ecological Reserve, one of the largest marine reserves in the world, creating a "no discharge zone" in the Florida Keys National Marine Sanctuary and developing partnerships with the cruise ship and marine industries to improve environmental management practices. Upgrading wastewater systems and acquiring land in the Florida Keys is also protecting the third largest barrier reef in the world.

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INTERNATIONAL SOCIETY FOR REEF STUDIES (ISRS)

Founded in 1980, ISRS is the largest society of reef scientists in the world with over 800 members. The principal objective of ISRS is to promote the production and dissemination of scientific knowledge and understanding of coral reefs, both living and fossil, for public benefit.

The ISRS produces Briefing Papers and Statements about the state of coral reefs for the benefit of the wider community. ISRS produces and distributes the quarterly scientific journal Coral Reefs containing peer-reviewed scholarly works on the geology, biology, ecology, and environmental issues regarding the world's coral reefs.

ISRS hold annual meetings, sponsors biannual conferences, and distributes the newsletter Reef Encounter, and supports students through fellowships.

ISRS sanctions the world's major coral reef science meeting, the International Coral Reef Symposium (ICRS), held every 4 years.

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C. Langdon, J. Kleypas, J. Horst

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Mini-Symposium 25: Predicting reef futures in the context of climate change: Is 500 ppm CO_2 and 2°C of warming the 'tipping point' for coral reefs?
O. Hoegh-Guldberg, A. Baker

Mini-Symposium 26: Biodiversity and diversification of reef organisms
A. Kerr, G. Paulay, and M. Pichon

EDITOR'S PREFACE

We proudly present you here with the Proceedings of the Eleventh International Coral Reef Symposium (11 ICRS), held at the Conference Center on Port Everglades in Fort Lauderdale, Florida between the 7th and 11th July 2008. The two proceedings volumes contain almost 300 contributions arising from this vibrant and well-attended conference – with nearly 3500 attendants the biggest one in the ICRS's proud history!

Papers encompass a wide variety of topics, stretching from geology, via biology into management science. The distribution of papers clearly reflects the evolving status of coral reef science in 2008. The sections with the most contributions are those concerning themselves with measuring the status of reefs, those dealing with resource extraction and management issues and those evaluating the impacts of man-made changes to the atmosphere and biosphere.

The load of the editorial process was carried by a great many people. The chairs of the individual mini-symposia reviewed the manuscripts in their sessions and then forwarded their recommendations to us. Those most active are named on the cover page, but most chairs carried at least some of the load. Their hard work is acknowledged on a separate cover page for each mini-symposium. Wendy Wood at NCRI spent a great deal of effort throughout the organizational process and also during final formatting, Amy Koch and Laura Checkett at Mira Digital Publications maintained the submission website and were instrumental in producing the paper and digital versions. We thank Dr. Jamie Oliver and Moi-Khim Tan of the World Fish Center for making the upload of this Proceedings to the ReefBase, and therewith its free dissemination, possible. We also thank the reviewers, too numerous to be named individually, for their crucial contribution.

Although we have done our best to produce an error-free Proceedings, undoubtedly many errors remain. We apologize for these. Hopefully the benefits of relatively rapid publication and the freedom given to authors to produce their papers as they themselves found most fitting, will outweigh the disadvantage. We hope that these Proceedings volumes will prove a valuable resource and carry forward the traditions begun in 1969 at the First such Symposium at Mandapam Camp in India. May many more follow!

Enjoy your Proceedings!

Bernhard M. Riegl
Richard E. Dodge

OUTCOMES OVERVIEW

Synopsis

A defining theme of the 11th International Coral reef Symposium was that the news for coral reef ecosystems are far from encouraging. Climate change happens now much faster than in an ice-age transition, and coral reefs continue to suffer fever-high temperatures as well as sour ocean conditions. Corals may be falling behind, and there appears to be no special silver bullet remedy. Nevertheless, there are hopeful signs that we should not despair.

Reef ecosystems respond vigorously to protective measures and alleviation of stress. For concerned scientists, managers, conservationists, stakeholders, students, and citizens, there is a great role to play in continuing to report on the extreme threat that climate change represents to earth's natural systems. Urgent action is needed to reduce CO_2 emissions. In the interim, we can and must buy time for coral reefs through increased protection from sewage, sediment, pollutants, overfishing, development, and other stressors, all of which we know can damage coral health.

The time to act is now. The canary in the coral-coal mine is dead, but we still have time to save the miners. We need effective management rooted in solid interdisciplinary science and coupled with stakeholder buy-in, working at local, regional, and international scales alongside global efforts to give reefs a chance.

Introduction

The 11th International Coral Reef Symposium concluded a marathon week (July 7-11, 2008) with over 3,500 attendees from 75 countries; 1032 oral and 1600 poster presentations; 26 mini-symposia; 3 special sessions; 20 field trips; addresses by 6 distinguished plenary speakers (Malcolm McCulloch, Joan Kleypas, Roberto Iglesias-Prieto, Robert Cowen, Drew Harvell, Daniel Pauley), the Darwin Medalist (Terry Hughes), the President of the International Society for Reef Studies (Richard Aronson), NOAA Administrator Vadm.Conrad Lautenbacher, and also by Florida's governor Charlie Crist, US Congressmen Ron Klein and Brian Baird, and Florida representative Ellyn Bogdanoff; 40 sponsorships from diverse government agencies (including Co-Sponsorships from the state of Florida, NOAA, and DOI), academic institutions, NGOs, and private industry; scores of exhibits; and a fine educational center.

The Local Organizing Committee, Super-Chairs of the 26 Mini-Symposia (scientific sessions) and President of ISRS provide this overview to highlight outcomes.

Coral Reefs Under Threat: Many Actions Can Help

Over a third of the world's coral species are at elevated risk of extinction. Reefs of the Western Atlantic have generally decreased in living corals since the 1970s, and although reefs of the Great Barrier Reef were resilient until 1996, they are now beginning to struggle. It is good news that reefs far from continents and direct human pressures, including Bermuda in the Atlantic; the Flower Garden Banks in the Gulf of Mexico; American Samoa, Fiji, Palau, and French Polynesia; and the islands of the Andaman Sea, Chagos,

and western Maldives in the Indian Ocean are still resilient and able to recover from damage. We still have some time!

The degradation and ponderous recovery of many reefs are due to combination of the global stresses of climate change coupled with regional and local stressors including runoff from agriculture, other land-based sources of pollution, over-fishing, and habitat destruction associated with coastal development.

Climate Change Effects of Warming and Acidification

Many studies have laid down stepping stones to understand ongoing changes in coral and fish community dynamics. A prime driving factor is climate change. NOAA satellites reveal tropical oceans have warmed at a significantly faster rate over the past decade. Coral cover in many locations remains low and is not recovering. This is especially true in areas that have experienced severe bleaching, a phenomenon on the increase with increasing ocean warming. Such changes are not restricted to corals. Coral loss clearly leads to loss of associated organisms; decreasing fish abundance and diversity, for example, go hand in hand with loss of corals and decreasing coral cover.

Ocean acidification and ocean warming can be thought of as the 'evil twins' of climate change. The same carbon dioxide that causes ocean warming is entering the oceans and causing chemical changes (i.e., lowered pH, lowered carbonate ion concentration) that affect marine life. Coral reefs are threatened because carbonate ions, essential for building their calcium carbonate skeletons, become less available. Reef structures are further endangered because lowered pH is likely to reduce resistance to erosion. In the naturally more acidic waters of the eastern Pacific, for example, reefs are less fortified by calcium carbonate cements and experience higher erosion rates than other reefs. It has been suggested that to save reefs, we cannot exceed 450ppm CO_2 in seawater. At the world's current rate of CO_2 emissions, we have 8-10 years to turn the tide.

There is mounting evidence that acidification can cause a slow down in the growth of corals and in coralline algae, both vital to the reef structure. Results presented at the Symposium show that calcification rates in many corals are already declining from a combination of factors including increasing temperature, decreasing water quality, and ocean acidification. In addition, ocean acidification is now shown to reduce the ability of coral larvae and coralline algae to successfully settle and grow new colonies, which will affect the ability of degraded reefs to recover through reseeding with larvae.

We can expect winners and losers in warmer and more acidic oceans, but the net result will not be stasis. Rather, it is likely that some species will not be able to maintain a foothold in the ecosystem, upsetting the normal day-to-day interactions between coral reef species, as well as the maintenance of the reef structure itself.

Scientific Advances

The Symposium witnessed many new science advances that increase our understanding about coral reef ecosystems. Several scientists used new genetic techniques to show that reefs are connected primarily at a scale of tens of kilometers (km). In contrast with previous thoughts that reefs were highly interconnected at scales of thousands of km, these studies show that only occasional pulses of propagules leave the reef 'village'. Coral larvae settle in close proximity to their parents, and some fish are homing in to local coral heads after tens of km of travel. Such local connectivity suggests that local protection and management will lead to local benefits. Action and protection also buys time for adaptation. Simultaneously we must not lose sight that important ecological connections and stressors (e.g., migration, larval transport, upwelling, pollution, pathogens, climate change) also exist at the larger ecosystem scale.

Molecular tools are revealing a diverse array of microorganisms living around and in association with reefs and able to adapt subtle environmental shifts. The microorganisms have vital roles in reef health, but if perturbed, these roles can break down in a variety of detrimental ways and lead to infection and disease. Researchers are reporting a link between coral disease and environmental change, and even viruses are now implicated in coral disease and associated coral tumors. Yet, there is also some evidence of developing immunity, whereby *Vibrio* and other bacteria that once caused harm to corals are no longer found in

association with the coral-disease. In addition, some Caribbean sea fans, seriously impacted in the first round of disease, seem to build up resistance.

Scientists are also looking at the genes that corals use to acclimate to surrounding conditions to determine how corals react to their environment. Corals can make their own sunscreen proteins and can customize their protection to light level by up- or down-regulating genes that control the protein production. Many other genes are also adjusted by corals in response to environmental cues that lead to spawning. There is a potential role in synchronization of spawning and corals may be 'talking' to each other, via chemical messengers, to help synchronize the final stages of spawning.

Some corals manage to thrive in harsh conditions such as extreme temperatures including tidal pools and in enclosed seas. Among various coping mechanisms, they may selectively cultivate particular types of symbiotic algae. The symbiotic algae that live inside corals power the latter's metabolism and skeleton formation. New insights show how the coral-symbiont relationship can improve stress resistance. Corals may be able to expel partners susceptible to heat or uv-stress, and up-regulate the population of more robust partners that were previously cryptic and barely detectable. These new algae keep the corals healthy at the higher than normal temperatures, or produce antioxidants to soak up toxic byproducts of increased chemical activity. The buildup of resilience of some reefs from previous hard knocks illustrates that we must protect not only beautiful reefs from abuse, but also the reefs in marginal conditions that may be less attractive at first sight. These might house the survivors for future generations.

Management

It is instructive that the largest of the 26 mini-symposia of the 11th ICRS was devoted to management topics. Related symposia concentrated on reef restoration, fisheries, and social ecological systems.

By 2015, half the world's population will live along a narrow band of coast, putting unsustainable pressures on coastal resources. There will be coral reefs in our future only to the extent that they are valued by people. Understanding the value of reef ecosystem goods and services can help to promote protection and thwart degradation. Economic benefits, incentives, and cultural values need to align with conservation efforts. Social scientists have much to contribute to this aspect as well as towards environmental use planning within local and regional areas

Likewise, managing reefs must include managing people's behavior toward reefs. Social science can help us understand the root causes of people's behavior, including poverty, global demand for fish products, and tourism. Rising food and fuel prices can put greater fishing pressure on near-shore reefs, while open access along the coasts of many poor countries is leading to a Tragedy of the Commons and serial depletion of fish stocks. Fisheries catches from many islands are vastly underreported, and fish populations severely depleted and in critical condition. But, through remote sensing, mapping, modeling, and decision support tools, it is possible for stakeholders to better visualize changes in reef resources, and policy makers to understand the impacts of decisions on the vital services that coral reefs provide.

Local action is one key method to achieving management success, and communities and government can be effective stewards of coral reefs once it is understood that it is in the interest of the public and individual good to do so. Linking coral reef conservation to human welfare, Healthy Reefs for Healthy People, is a useful theme to be repeated with ties to tourism, livelihoods, food security, as well as cultural and spiritual well being.

Marine Protected Areas are multiple-use areas, in which various tools, particularly zoning, are used to protected marine resources (marine reserves or no-take areas), define and confine human uses, separate conflicting activities, and consolidate administration to eliminate duplication and overlap. Studies in the Philippines and Guam, for example, show that corals inside MPAs have less disease than fished areas which have large gaps in diversity affecting affect disease transmission. MPAs represent effective management tools and work best when providing interim benefits as well as longer conservation benefits. Scientists reiterated that MPAs are necessary but not sufficient. Protection of as much reef territory as possible (ecosystem based management) recognizes that local efforts can build to regional ones which in turn can build into global ones. Organizational and scientific capacity is needed to establish and maintain

marine management areas. In some cases, hybrid designs of no-take and periodically closed areas may better address economic needs of the community and conservation objectives.

Restoration techniques are also advancing and offer promise especially where the original cause of the reef decline has been removed or addressed.

Many stressors including overfishing, run-off, and development must be addressed. A village approach, natural and social scientists, managers and local communities working together, could make it happen. Changes needed to protect the word's coral reefs include: 1) convincing communities that reefs are at risk, 2) enlisting multiple levels of public, industry and government support, 3) providing necessary legislative and regulatory powers, and 4) conducting needed science with sufficient information transfer. In this way effective management rooted in broad interdisciplinary science and stakeholder buy-in, working at local, regional, and international scales, coupled with global efforts to reduce atmospheric CO_2, will give reefs a chance.

The 11th ICRS Local Organizing Committee	**Super Chairs of the Mini-Symposia**	**President of ISRS**
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A Call to Action for Coral Reefs

2008 is a critical time for coral reefs. At the 11th International Coral Reef Symposium held in July, midway in the International Year of the Reef, over 3,500 experts from 75 countries assembled to face some hard truths: coral reefs are teetering on the edge of survival, and it is our fault. High levels of carbon dioxide in the atmosphere can produce a lethal combination of warmer seawater and lower pH. Pervasive overfishing, pollution, coastal development, and physical damage further undermine reef health, and consequently that of the people and ecosystems depending upon them. An overview of the 2632 papers presented can be found on http://www.nova.edu/ncri/11icrs/outcomes.html

Coral reefs feed, protect, and provide livelihoods for hundreds of millions of people around the world. They create homes for billions of fish and other animals, buffer coastlines from the ravages of storms, and provide rich economic opportunities through tourism and fishing. Their value to society has been estimated at more than $300 billion/yr. Reefs are the dynamic centers of the most concentrated biodiversity on Earth. Losing coral reefs would rob the world of one of nature's most precious gifts.

Despite these challenges, it is not too late to save coral reefs. The 11th ICRS gave a renewed sense of purpose and hope for the future. A consensus emerged that society has both the knowledge and the tools to bring coral reefs back from the brink. The only question is – will we act? We have a real—but rapidly narrowing—window of opportunity in which to take decisive action. We must immediately:

- **Cut CO_2 emissions by lowering our carbon footprint and ask our policymakers to commit to low carbon economic growth.**
- **Eliminate open access fisheries in coral reef ecosystems.** Establish and enforce regulations on user rights, total allowable catch, individual catch quotas, non-destructive gear and other sustainable fisheries regulations.
- **Protect coral reef herbivores, including parrotfish.** Ban the harvesting of these species for sale and commercial consumption.
- **Establish and strictly enforce networks of Marine Protected Areas that include No-Take Areas**. Consult with local communities and authorities on design and benefit sharing to maximize returns and build sustainability into the process in order to protect marine biodiversity and restore vital fish stocks.
- **Effectively manage the waters in between Marine Protected Areas.** The enforcement of coastal zoning, environmental impact assessments and 'polluter pays' regulations can help control marine and land-based sources of pollution, while strategic environmental assessment can effectively manage coastal development and tourism.
- **Maintain connectivity between coral reefs and associated habitats**. Mangroves, sea grass beds and lagoons contribute to the integrity of reef ecosystems and their continued production of ecosystem services.
- **Report regularly and publicly on the health of local coral reefs**. Include assessments of the effectiveness of management and conservation measures.
- **Recognize the links between what we do on land and how it affects the ocean**. We live on a blue planet—our health depends on ocean health.

- **Bring local actors together to develop a shared vision of healthy reefs and a road map for getting there.** Engage members of industry, civil society, local government and the scientific community to set ambitious targets and performance indicators.
- **Work for change with management to produce desired outcome.**

Only by taking bold and urgent steps now can we hope to ensure that reefs will survive to enrich life on earth, as they have for millions of years before us. By failing to act we risk bequeathing an impoverished ocean to our children and future generations.

Sincerely,

Richard E. Dodge, Chair, **11th ICRS Local Organizing Committee**

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PROCEEDINGS OF THE

Mini-Symposium 1:

Lessons from the Past

Convened and edited by:

D. Hubbard, W. Ramirez, E.A. Shinn, L. Greer, C. Sherman, G. Stanley

Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008
Session number 1

A New Caribbean Reef Model: A View From Ye Shoulders of Giants

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Abstract. For geologists, three cores from Lang Bank described at the 1977 ISRS meeting in Miami, FL set the direction of the coral-reef discussion for the next three decades. High accretion rates from this and other Caribbean sites led us to ask why reefs capable of outpacing even the fastest sea-level rise could be abandoned. A possible gap in accretion on Lang Bank at 10,000 CalBP attributed to dirty water flowing off the recently flooded bank provided a solution to this "drowning paradox", and the Lang Bank story was extrapolated to the entire Caribbean. Recent studies suggest that some of the foundational ideas upon which our models were built may warrant reexamination. *Acropora palmata* reefs not only continued to build, but thrived across the presumed Caribbean-wide gap. Caribbean reef building can generally be characterized as transgressive between 11,000 and 7,000 CalBP due to a steady and rapid rise in sea level, and regressive thereafter, as sea-level rise slowed to below 4 m/Kyr. However, two millennial-scale lapses remain for *A. palmata*, starting at ca. 6,000 and 3,000 CalBP, respectively, well after sea-level rise had slowed to below the average rate of reef accretion. Their origins remain unknown, but could bear on the species' recent decline.

Key words: Caribbean; Reef Models, Reef Accretion, Holocene, *Acropora*

Introduction

Our earliest coring studies were designed primarily to address Darwin's subsidence hypothesis. The reef and underlying limestone intervals atop the epic cores through the Marshall Islands (Ladd and Schlanger 1960) were catalogued as "reefal limestone" and set in storage once the basalt intervals that vindicated Darwin were encountered. This focus was changed by the development of a small and cost-effective drilling system that provided access to the interiors of modern reefs (Macintyre 1975). Early descriptions for the Caribbean (Adey 1975; Macintyre and Glynn 1976; Shinn 1980) were quickly followed by studies along the Great Barrier Reef (Hopley 1982; Davies et al. 1985), the Indo-Pacific region (e.g., Camoin et al. 1997; Montaggioni et al. 1997), and numerous other locales throughout the tropics.

At the Coral Reef Symposium in Miami, Adey et al (1977) described reef accretion along the southwestern corner of Lang Bank east of St. Croix in the US Virgin Islands (Fig. 1). *Acropora palmata* dominated early shelf-edge reefs that built rapidly until ca 10,000 CalBP, when the reefs suddenly died off. Adey et al proposed that the sudden demise was caused by turbid water flowing from the recently flooded bank. Water depth over the failed reefs increased as sea level rose over the ensuing 2,000 years. As the waters cleared, massive corals dominated the new reef community, now in water too deep for branching acroporids. The sequence of events at Lang Bank provided the foundation for Caribbean-wide models of Holocene reef development (Adey 1978; Macintyre 1988) that

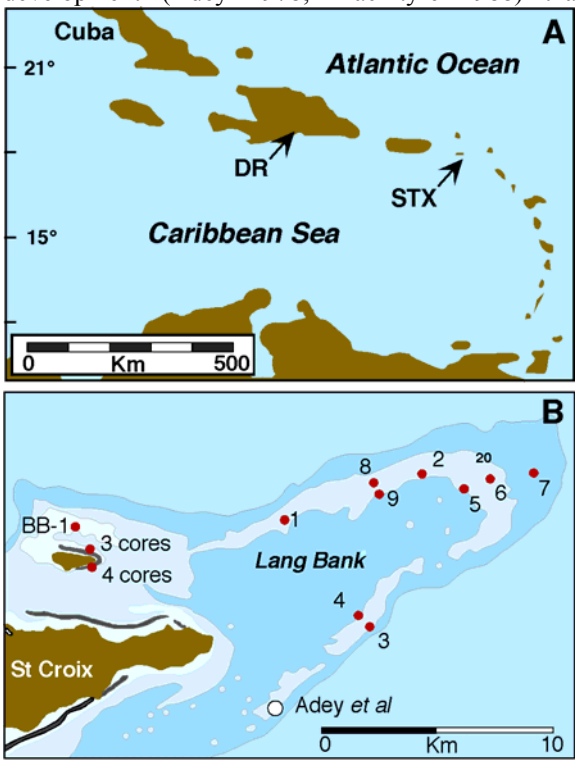

Figure 1: Location of cores (closed circles) on Lang Bank. TheSmithsonian core site described in Adey et al (1977) is shown by the open circle.

envisioned "inimical bank waters" related to platform flooding creating similar scenarios throughout the region.

This paper describes more recent core data from Lang Bank and elsewhere across the Caribbean. These data show that the interval of non-accretion within the early Lang Bank cores was largely absent elsewhere on the bank and the larger Caribbean region. An alternative and simpler Holocene Reef model based on the rate of sea-level rise and moderated accretion reef rates is proposed.

Material and Methods

On Lang Bank, four cores (LB1-LB4: Fig. 1) were recovered using a small drilling system similar to the one described by Macintyre (1875). Five additional cores on Lang Bank and eight from Buck Island were recovered using the SCARID Drilling System. In separate studies, cores were recovered from shelf-edge environments off SW Puerto Rico (Hubbard et al. 1997) and Florida (Toscano and Lundberg 1998). Cores were recovered in 1.5-m intervals, and notes during drilling permitted logging accuracy within a few centimeters. Samples were slabbed longitudinally and corals were identified to species level. Fresh-looking samples were used for radiocarbon dating, following XRD analysis to confirm their pristine nature. Methods are described in more detail in Hubbard et al. (2005).

Results

The Lang Bank cores provide a record of vigorous reef accretion around Lang Bank starting ca. 11,000 years ago (oldest ages are from Adey et al. 1977), and continuing until ca 5,000 CalBP (limited to massive corals after 6,300 CalBP). Along the northern platform margin, *A. palmata* reefs started up later (ca. 8,100 CalBP), a timing consistent with the scenario proposed by Adey et al. (1977). However, at core site LB-3 located east of the Smithsonian site (Fig. 1) reefs dominated by *A. palmata* started as early as 9,250 CalBP (i.e., in the middle of the proposed hiatus: Fig 2), and continued to accrete until 6,400 years ago. In core LB-6 on the eastern end of the bank, there is over 5 meters of reef between the Pleistocene surface and a sample that dated at 8048 ± 70 CalBP (Table 1). It therefore seems likely that both *A. palmata* and massive-coral reefs along at least the southern and eastern margins continued to build through the 10-8 Kyr interval when inimical bank waters were previously assumed to have shut down reefs all around Lang Bank.

If we examine reefs elsewhere in the Caribbean, similar patterns emerge. Reefs along the southern shelf edge of nearby Puerto Rico (e.g., PAR-11: Fig.2) were similarly dominated by *A. palmata* and

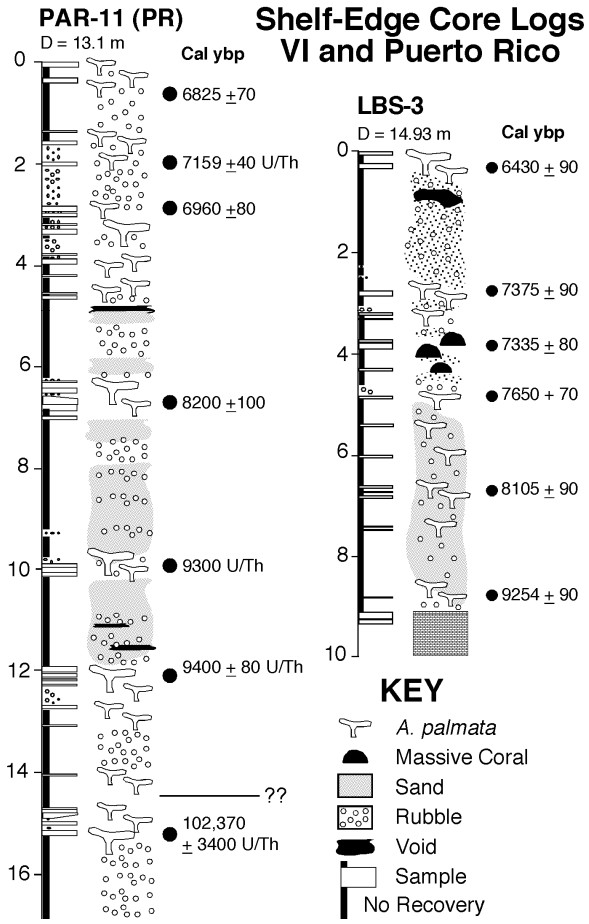

Figure 2: Core logs from the shelf edge of Lang Bank and SW Puerto Rico. Note the abundance of *A. palmata* over the interval between 10,000 and 8,000 CalBP when reefs quit at the Adey et al site. The left column shows recovery. The right log is interpreted.

continued to build through the proposed gap that was subsequently incorporated into the Caribbean reef models of Adey (1978) and Macintyre (1988).

Figure 3 summarizes the occurrence of *A. palmata* reefs over the past 11,000 years. The data are derived from a variety of water depths and reef types across a wide geographic range within the Caribbean. The number of dated *A. palmata* samples within Box A shows no significant decrease in the density of the branching species between 10,000 and 8,000 CalBP. Furthermore, *A. palmata* remained as an important component of Caribbean reefs immediately after 8,000 CalBP.

Discussion

Ian Macintyre's submersible drill provided our first real opportunity to chronicle Holocene reef history. Starting with the hallmark Panama study (Macintyre and Glynn 1976), coring investigations spread first across the Caribbean and eventually to every major tropical ocean. The earlier studies focused on the

Table 1. Lang Bank Core Statistics. All age errors are less than 100 years.

Core	Oldest	Youngest	Corals	Comments
1	6476	6215	Mixed	Not to Pleistocene
2	8075	6625	*A. pal*	To Pleistocene
3	9250	6340	*A pal*	To Pleistocene
4	8060	5885	Mixed	Not to Pleistocene
5	6740	6130	Mixed	Not to Pleistocene
6	8050	4860	Mixed	5m to Pleistocene
7	6615	5035	*A. pal*	To Pleistocene
8	7310	6350	A pal	To Pleistocene
9	7145	6350	Mixed	Not to Pleistocene

internal fabric of modern reefs, with an eye toward comparisons with their ancient counterparts. The Smithsonian study on SW Lang Bank was a turning point inasmuch as it looked, for the first time, at the relationships between processes and products – relating larger-scale patterns of reef development to both local (sedimentation) and global (sea level) controls.

Citing reports of accretion rates near 10m/Kyr, Schlager (1981) argued that it was problematic that shallow-water reefs, most of which were capable of building at rates faster than the rise of Holocene sea level, could have been abandoned. He resolved this "drowning paradox" by invoking either sudden (and usually short-lived) jumps in sea level or a local deterioration of water quality sufficient to slow or kill corals. The Lang Bank story provided the best example of the impact of "inimical bank waters" related to the flooding of long-exposed shelves.

It was further argued that carbonate banks existed at similar depths throughout the Caribbean, and the scenario of early start up by *Acropora*-dominated reefs, followed by abandonment as platform tops flooded could be generalized to the region. From this, it was proposed that high-energy Caribbean shelf margins would host "relict *give-up reefs* along the upper slopes and shelf edges, and relatively young late Holocene reefs fringing most coastlines" (Macintyre 1988).

More recent data from this and other Caribbean sites do not support this scenario. Recent compilations of reef-accretion rates throughout the Holocene (Indo-Pacific and Indian Oceans: Dullo 2005; Caribbean: Hubbard 2008) show that accretion rates near 10m/Kyr are the exception and that most reefs built at half this rate or below. Hubbard (2008) computed an average accretion rate for Caribbean reefs of 3.5 m/Kyr. The long-assumed decrease in accretion with depth was not supported by the data. Furthermore, the accretion rate for reefs dominated by branching *A. palmata* (3.83 m/Kyr) was not significantly different from the rate of building for reefs with mostly massive corals (3.07 m/Kyr). This was tentatively

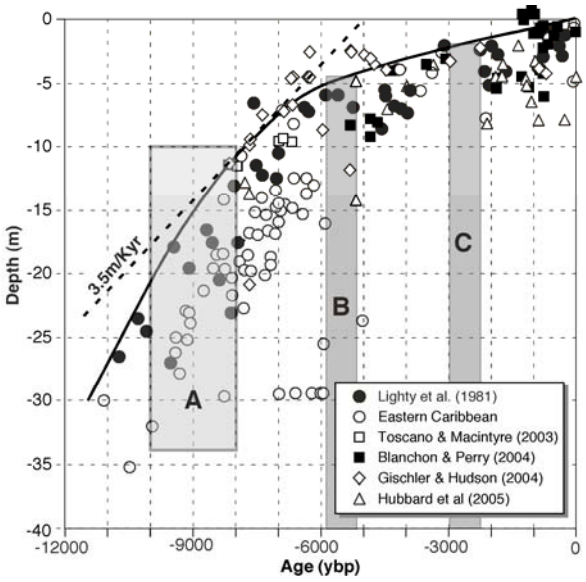

Figure 3: Summary of depth and age data for *A. palmata* in Caribbean cores (sources are provided in the legend). Box A outlines the interval across which *A. palmata* are supposed to be absent based on existing Caribbean reef models. Note that *A. palmata* not only survives but thrives across this predicted gap. Shaded intervals B and C represent times for which dated *A. palmata* samples are either rare or absent. While *A. palmata* reefs have generally occurred since 11,000 years ago, those on the left side of Box B sit near shelf margins and have not been active since ca. 6,000 years ago. Those to the right are shallower reefs, closer to shore, throughout the region. The two millennial-scale intervals of poor *A. palmata* development both occurred after sea-level rise had slowed, and some other mechanism must be invoked to explain the apparent difficulty that this rapidly growing species had encountered. The dashed line represents the average Caribbean reef-accretion rate proposed by Hubbard (2008) and intersects the sea-level curve at ca. 7,500 CalBP. Before this time, reefs would have had a more difficult time keeping up with rising sea level than after sea-level rise slowed. Modified from Hubbard et al (2005).

explained by depth-related patterns of bioerosion and down-slope transport that mirrored calcification.

If we discount the anomalously high rates cited by Schlager (1981) as typical of reef accretion, then a simpler model emerges – one that does not involve a "drowning paradox" or a need to resolve it. Figure 4 illustrates the accretion patterns for several reefs on St. Croix and Puerto Rico. Apart from a few examples, reef accretion was close to 3.5 m/Kyr and was independent of the coral species recovered in the cores.

Based on accretion near 3.5m/Kyr, early shelf-edge reefs were building at rates slower than sea-level rise. Presumably these were initially being outpaced. However, the rise of sea level was gradually slowing, and by 7,500 years ago sea-level rise and accretion were roughly balanced (dashed line in Figure 3). After this time, the balance between sea-level rise and accretion gradually reefs tipped in favor of the reefs.

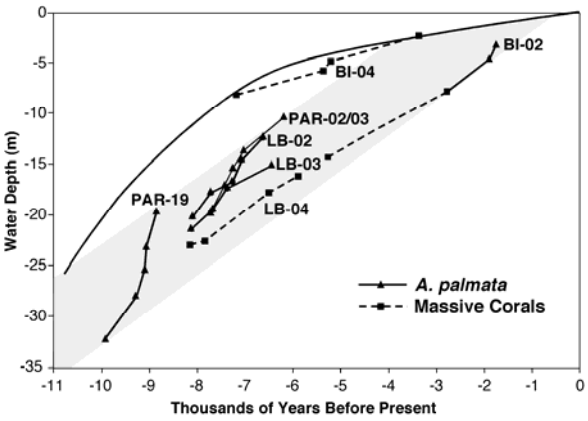

Figure 4: Accretion curves for several cores from St. Croix and Puerto Rico. These data are for single cores contained within the data base used to construct Figure 3, and most other reefs exhibited similar accretionary histories. Note that the apparent gap between ca. 8,500 and 8,000 CalBP is an artifact of the cores chosen to illustrate the accretionary pattern of eastern Caribbean reefs. Other than the deeper reef off Puerto Rico (PAR-19) and the reef constrained by sea level (BI-04), reef accretion generally conforms to the average Caribbean rate of 3.5 m/Kyr (shaded envelope). The dashed line in Figure 3 corresponds to this rate and intersects the Caribbean sea-level curve at ca. 7,500 CalBP.

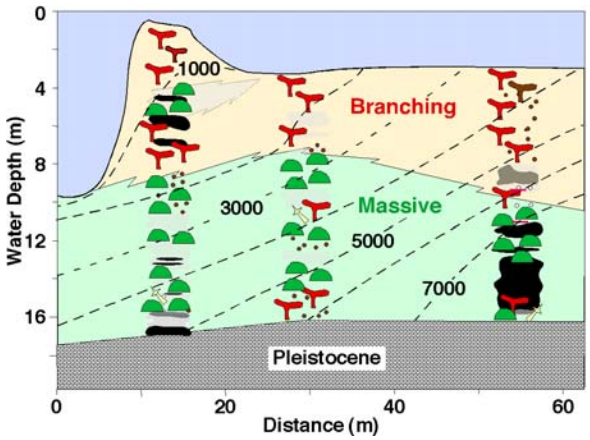

Figure 5: Cross section through the northern reef at Buck Island. Throughout its history, this reef had an accretionary potential that was greater than accommodation space being created by slowing sea-level rise. As a result, the reef built not only upward but also seaward. The initial position of this reef was roughly midway across the lagoon at a point not on this cross section. Thus, the reef has built more than 60m seaward over the past 8,000 years. Modified from Hubbard et al. (2005).

All the Caribbean shelf-edge reefs cored to date remained active after 7,500 CalBP. Based on their initial accretion rates, they should have caught up with rising sea level between 6,000 and 3,000 years ago.

Reefs that are presently emergent sit closer to shore and started up by 8,000 CalBP. Their emergence reflects the fact that sea level rose close to or slower than the reefs built. Those reefs that were at sea level (e.g., core BI-04 from Buck Island: Fig. 4) were producing carbonate faster than accommodation space was being created. As a result, they built now only upward but also seaward. At Buck Island (the small island north of St. Croix in Figure 1b) the reef that rims the eastern half of the island built seaward by more than 60m over the past 8,000 years (Fig. 5). Similar patterns have been documented for Tague Reef on northeastern St. Croix (Burke et al. 1989).

Holocene reefs exposed in the western Dominican Republic (Fig. 6) provide a unique opportunity to examine the facies architecture in much greater detail than is possible from cores. Along a 450-m long outcrop, the older section (10,000 - ~6,000 CALBP) reflects a deepening-upward sequence. In the deeper and older part of the outcrop, shallow tidal molluscs give way to massive corals and finally platy corals found at depths over 25 m on modern reefs. The shallower end of the transgressive facies bundle yielded dates as young as 5,700 CalBP (Fig. 6b), after sea level had started to slow down. Facies still deepen upward (branching → mixed coral facies). Higher (and younger) in the outcrop, the pattern is reversed, and the shallow-water branching facies builds out over the deeper mixed-coral facies (Fig. 6c) in response to even slower sea-level rise. This is analogous to the conditions seen in Figure 5 for Buck Island in the US Virgin Islands.

Collectively, these examples illustrate a simpler model for Caribbean coral-reef accretion (Fig. 7). Prior to ca. 7,500 CalBP, sea level was rising faster than shelf-edge reefs could build and they tended to gradually lag behind. By the time sea level slowed, most of them were still in water depths favorable to *A. palmata*, and reef-building gradually matched and eventually exceeded the rate of sea-level rise. Reefs closer to shore started later (9,000 – 7,000 CalBP) and in shallower water. As a result, they were able to either keep up or catch up to sea level, which they have successfully tracked ever since. As sea level continued to slow and accommodation became increasingly inadequate, these reefs have generally built both upward and seaward.

One problem emerges from all of this. As discussed above, shelf-edge reefs throughout the region should have been able to reach sea level by 4-3,000 years ago. For reasons that remain unexplained, every shelf-edge reef that has been sampled stopped accreting between 7,000 and 6,000 CalBP. This includes reefs from Lang Bank (this study), SW Puerto Rico (Hubbard et al. 1997), northern Florida (Lighty et al. 1982) and the Florida Keys (Toscano and Lundberg 1998). This occurred well after sea level slowed, and some other mechanism must be invoked – and it recurred at ca. 3,000 CalBP, again for unknown reasons. The question remains whether/how this might bear on the recent decline of *A. palmata*.

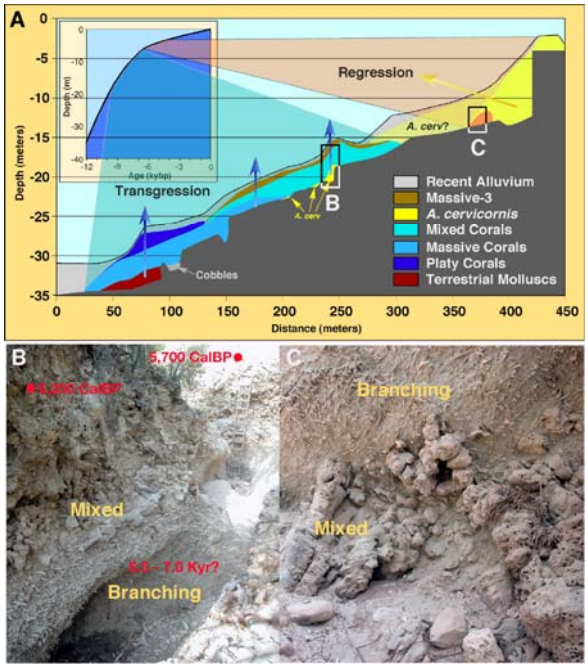

Figure 6: Facies distribution in Cañada Honda, western Dominican Republic. A) Cross section along the outcrop showing the facies distribution. Note that in the lower part of the outcrop, environments deepen upward (massive→ mixed), while in the upper outcrop the reverse occurs. Photo locations for B and C are indicated. B) Photo looking up-canyon. The sequence still deepens upward shortly after sea level has slowed down. C. Photo of a younger section near the upper end of the outcrop. Note that the section shallows upward as the branching *A. cervicornis* facies builds out over the deeper mixed-coral facies. This shift from transgressive to regressive facies architecture reflects the slowing of sea-level rise and the outbuilding of the younger section of the reef, similar to what was seen in the Buck Island reefs (Fig. 5). For outcrop location, see "DR" in Figure 1.

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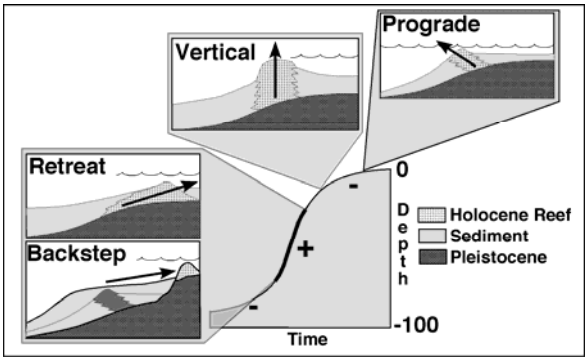

Figure 7: Facies model for Holocene sea-level rise. Prior to 7,500 Cal BP, sea level was rising at a rate faster than average accretion (+ on the curve). In this scenario, reefs either retreated upslope, backstepped (Hubbard et al, 1997) or drowned. As sea-level rise slowed around 7,500 CalBP, reefs first built vertically as accretion matched sea-level rise, and eventually started to build out over deeper mixed- and massive-coral facies (Figs. 5, 6). Presently emergent reefs closer to shore initiated as sea level was slowing, and accretionary potential quickly exceeded accommodation space. As a result, they have typically built seaward over their history.

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Response of *Acropora* to warm climates; lessons from the geological past

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Abstract. There is currently widespread concern about the deterioration of living reef corals, such as *Acropora*, and tropical reefs. Much of their demise appears to be related to coral bleaching, the underlying cause is probably global climatic warming. Future predictions about the responses of modern coral reefs lacks data from the geological past. The fossil record shows reef coral distributions are highly sensitive to climatic change, modulated by the availability of habitat. Here we follow the history of one individual taxon, *Acropora*, to demonstrate how a particularly important reef coral genus has responded to global change through its geological time-span. *Acropora* is the most diverse, widespread and abundant of today's tropical reef corals, with its centre of diversity in the Indo-West Pacific. Counterintuitively, it was previously absent from this region but was common in the Paleogene to early Neogene in Europe, including high palaeolatitude (48˚N) Eocene occurrences of southern England and northern France. We have assessed a unique set of unaltered, but fragmented, specimens of *Acropora* from the Eocene of the Paris and Hampshire basins for their preservational state, diagenesis, and stable isotopic composition ($\delta^{18}O$ and $\delta^{13}C$). Results suggest post-mortem parautochthonous to autochthonous deposition in low-energy embayments, with rapid burial in silty muds creating a relatively enclosed geochemical system. Taxonomic uniformitarianism and stable isotope data confirm that *Acropora* existed in tropical-like climatic conditions in Northwest Europe during the Eocene.

Key words: *Acropora*, Eocene, Hampshire Basin, Paris Basin, climate change.

Introduction

Global climatic changes, combined with regional anthropogenic processes, are combining to put modern coral diversity and distribution under threat (Hughes et al. 2003). It appears that corals are responding to such changes through acclimatisation and adaptation (Hoegh-Guldberg 1999) to occur in a broader latitudinal occurrence belt. In order to fully understand and predict the type, and magnitude, of future responses of coral reef ecosystems it is necessary to understand these changes from the geological record. Within this record, are data on the effects of both long-term and rapid climatic changes on coral reef distribution and diversity. This therefore provides a baseline, and possible analogues, to aid predictions about the future changes to coral reef ecosystems.

Much of our understanding of coral reef ecosystems from the geological past is based on taxonomic uniformitarianism, but by combining this with independent data it is possible to make higher resolution predictions about future responses. The aim of this paper is to add palaeoenvironmental context to high-latitude Eocene occurrences of the reef-building coral genus *Acropora* in order to test hypotheses on the effect of an Eocene phase of climatic warming in Northwest Europe. *Acropora* is the most successful of today's tropical reef-building corals and is presently found within the modern coral limits of ~32º north and south of the equator. The genus is found within all three major oceans of the world with a greatest diversity in the Indo-Pacific oceanic realm. Counterintuitively, *Acropora's* fossil record indicates that it originated and diversified in a North African-Mediterranean region (Wallace & Rosen, 2006, White et al., 2006, Wallace, 2008) with oldest records from the late Paleocene of Italy (Moussavian and Vecsei 1995), Austria (Tragelehn 1996) and Somalia (Carbone et al. 1994).

The localities (Fig. 1) in the Hampshire and Paris basins represent the most northerly latitudinal extent (~48ºN) of *Acropora* in its entire history. These high latitudinal occurrences coincide with a period of global warming in the Middle Eocene (Zachos 2001). During the Eocene, the Hampshire and Paris basins formed part of a larger intracratonic basin system which spanned much of southern England, the English Channel and northern France (Curry 1992).

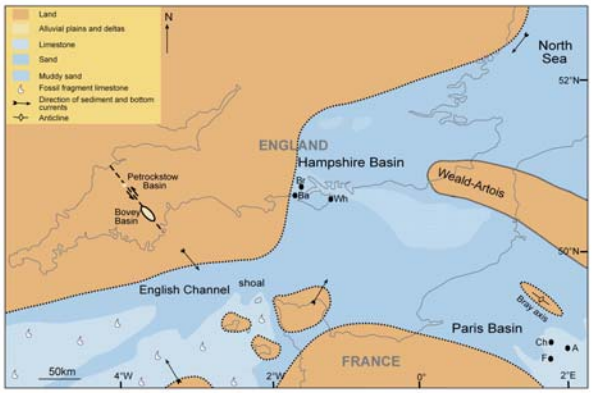

Figure 1: Palaeogeographic setting of the Hampshire and Paris basins in the Middle Eocene (Br: Brockenhurst, Ba: Barton, Wh: Whitecliff Bay, Ch: Chambers, A: Auvers sur Oise, F: La Ferme de L'Orme) (Adapted from Cope et al., 1992, & Gale et al., 2006)

The basins existed as two interconnected, shallow-marine, paralic embayments, connected to the Atlantic and with an intermittent connection to the North Sea. Cenozoic tectonic activity within the two basins included monoclinal and anticlinal folding, resulting from movement on deep-seated faults reactivated during Eocene Pyrenean compression (Lacombe and Obert, 2000). Episodic uplift of these structures led to remobilisation of Mesozoic and Cenozoic clastic sources and the creation of islands within each basin. The formation of structures such as the Weald-Artois anticline, combined with later Eocene global cooling, led to the intermittent isolation of the two basins from the North Sea followed by their Late Eocene restriction and closure. Hence the existence, both temporally and spatially, of *Acropora* in the two basins was limited and modified, not only by global, but regional and local tectonic and climatic factors.

Materials and Methods

Localities

Six localities identified by Wallace and Rosen (2006) were reviewed in this research: two Priabonian sites (Whitecliff Bay and Brockenhurst in England), two Bartonian (Auvers-sur-Oise in France and Barton in England), and two Lutetian (Chambors and La Ferme de l'Orme in France) (Fig. 1). Pristine *Acropora* specimens were available for geochemical analysis from 3 of these localities (Brockenhurst, Auvers-sur-Oise and Chambors) in the Hampshire and Paris Basins. Various sources were used for recent interpretations of the bio-, litho- and magnetostratigraphy for each locality (Aubry 1985, Gely and Lorenz 1991). Stratigraphic and geographic data is summarised in Table 1.

Fieldwork within both basins has shown *Acropora*-bearing lithologies are dominated by fine sands and muds. The associated molluscan fauna in both basins is diverse, abundant and dominantly marine although in some Hampshire Basin lithologies some of the fauna has a estuarine affinity. Other notable fauna includes sharks teeth in the Whitecliff Bay, Brockenhurst Bed and *Nummulites* in the Auvers, Sables d'Auvers Bed. Associated fauna are well preserved and have few limited borings. Reworked pebbles are found within both basin lithologies.

Specimens

71 fossil *Acropora* fragments were studied, with 11 of these used for geochemical analysis with an additional 8 of the genus *Lobopsammia* from *Acropora*-bearing lithologies. Five of the *Acropora* specimens were identified as *Madrepora*, the generic synonym extensively used by nineteenth century authors (Dana 1846). Most of the specimens are labelled as the fossil species *Acropora solanderi* (Defrance), with the rest as non-specific *Acropora.* Six of the *Lobopsammia* specimens were identified as the fossil species *Lobopsammia cariosa* (Goldfuss), with one identified as non-specific *Lobopsammia.* All *Acropora* specimens were re-identified on the basis of the taxonomic review of fossil *Acropora* (Wallace and Rosen 2006; Wallace 2008). From the Hampshire

Locality	Palaeolatitude[1]	Age (Ma)[2]	Stratigraphic age[2]	Biostratigraphic age[2]	Lithostratigraphy[3]	Position within stratigraphy[3,4]	Facies[4,5]
Brockenhurst, Hampshire, England	48.4ºN	36.0-34.2	Priabonian, Late Eocene	NP 19-20	Brockenhurst Bed	Base of Colwell Bay Member (formely Middle Headon Beds), Headon Hill Formation, Solent Group	Mainly composed of muds, sandy muds and muddy sands
Whitecliff Bay, Isle of Wight, England	48.3ºN	36.0-34.2	Priabonian, Late Eocene	NP 19-20	Brockenhurst Bed	Base of Colwell Bay Member (formely Middle Headon Beds), Headon Hill Formation, Solent Group	Mainly composed of muds, sandy muds and muddy sands
Barton, England	47.5ºN	40.4-37.2	Bartonian, Late Eocene	NP 16-17	Barton Group	Above the Bracklesham Group and overlain by the Solent Group	Fine sands and muds
Auvers-sur-Oise, France	46.9ºN	43.4-40.4	Bartonian, Late Eocene	NP 16	Sables d'Auvers	Base of the Sables Moyen, Auversian	Sands of variable character
Chambors, France	46.5ºN	47.3-43.4	Lutetian, Middle Eocene	NP 15	Calcaire Grossier (Upper)	Upper Middle to Upper Lutetian deposits, Abrard Zone IVa	Fine sands and muds
La Ferme de l'Orme, France	46.1ºN	47.3-43.4	Lutetian, Middle Eocene	NP 15	Calcaire Grossier (Middle & Upper)	Middle to Upper Lutetian deposits, Abrard Zone III & IVa	Fine sands and muds

Table 1: Summary of coral-bearing locality information from; 1. Paul Markwick (GETECH, University of Leeds), 2.Gradstein et al., 2004, 3. Various authors, 4. Fieldwork, 5. After Wallace & Rosen, 2006.

Basin localities 3 species were identified (*Acropora anglica* (Duncan), *A. bartonensis* (Wallace), *A. roemeri* (Duncan)) and from the Paris Basin 6 species were identified (*Acropora alverezi* (Wallace), *A. deformis* (Michelin), *A. ornata* (Defrance), *A. protacea* (Wallace), *A. solanderi* (Defrance), *A. wilsonae* (Wallace)).

Methods

Three approaches were used. Firstly a thorough literature review provided information about the global, regional and local palaeoenvironmental context of *Acropora* in the Eocene Hampshire and Paris basins. Secondly, fieldwork at selected locations provided detailed sedimentological and palaeoenvironmental data. Finally, a variety of quantitative and qualitative techniques (hand & thin section microscopy, x-ray diffraction, cathodoluminescence, scanning electron microscopy and stable C and O isotope analysis), allowed an assessment of preservation and diagenesis, which independently aided the palaeoenvironmental interpretation. Fossil corals often undergo post-depositional diagenesis during which secondary minerals and/or cements are deposited. The inclusion of these in any analysis may significantly affect the bulk coral trace element geochemistry of the sample leading to erroneous estimates of past climate. Petrographic and XRD enabled selection of pristine unaltered skeletal aragonite for stable isotope work.

Stable isotope analysis of $\delta^{18}O$ and $\delta^{13}C$ ratios in carbonates enabled inferences to be made about changes in environmental conditions with oxygen isotope ratios reflecting variables such as temperature and global ice-volumes (e.g. Patterson 1998; Tripati et al. 2004), while the isotopic signature of carbon may be used as a proxy for metabolism and reproduction (Patterson 1998; Patterson 1999). Powdered carbonate samples were obtained using a computer controlled micromill with samples being taken along linear transects along the axial corallite. Stable isotope mass spectrometry was performed at University College London using a Thermo Delta GASBENCH Mass Spectrometer. Isotope values are reported in per mil V-PDB (‰) with respect to modern ocean water (‰).

Results

Morphology and taphonomy

A comparison between the preserved morphological features of a modern and Eocene *Acropora* specimen confirmed the exceptional preservation of the Eocene specimens. Thin section and scanning electron microscopy of fossil specimens identified intact morphological features including axial corallites, radial corallites and coenosteum (Fig. 2a & b). All specimens show axial corallite apices with some distinguishable radiating septa. Radial corallites are identifiable with variations in costate or reticulate coenosteum (Fig. 2c & d). Selected specimens show radial corallites abraded flat against the main wall structure (Fig. 2b). In thin section multiple pristine microstructures were identified (Fig. 2a). Primarily, the aragonite composition is shown by the brown colouration of the skeleton with fanning, elongate trabeculae. The coenosteum exhibits a porous nature with large void-rich areas. Axial and radial corallites are recognised by large elongate voids and elongate septal remnants, particularly in the axial corallites.

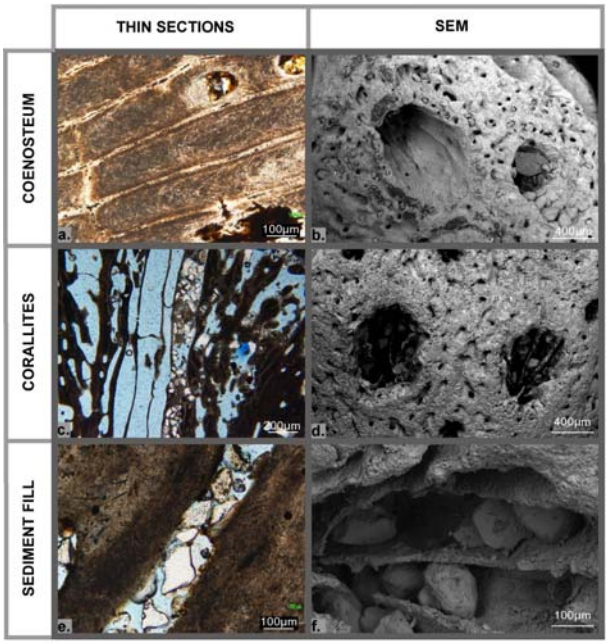

Figure 2: Summary of preservation and diagenetic features identified using thin sections and SEM. a. Characteristic trabeculae structure of skeletal aragonite. b. Highly porous coenosteum with sediment infilling. c. Axial corallite identified by elongate septal remnants and void areas. Fine grained sediment infilling corallite, mostly subangular-angular monocrystalline quartz. d. Radial corallites with remanent septal structure and sediment infilling. e. Fine grained sediment infilling void areas. f. Sediment fill within septal structure of a radial corallite.

Due to the exceptional preservation and limited fragmentation of some of these specimens, evidence of colonial types can also be seen. The Hampshire Basin specimens show variations of the four main colonial growth forms; tabular, arborescent, digitate and hispidose (Wallace 2008), and in the Paris Basin three growth forms; arborescent, digitate and tabular. Another morphological distinction between the specimens in each basin is the branch thickness. Specimens of *Acropora anglica* and *A. roemeri* from the Hampshire Basin have a sturdier growth form than species from the Paris Basin, with an average branch

thickness of 1.16 cm and 0.79 cm respectively for all species found (Fig. 3). Another significant difference is the larger amount of infilling sediment in the Hampshire Basin specimens probably reflecting the finer grained nature of the lithology from the Hampshire Basin and the larger void structures produced by the larger, coarser skeletal structures in specimens.

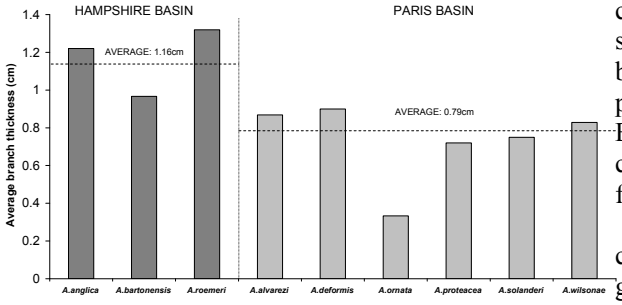

Figure 3: Variation in branch thickness shown between each species, and overall between the two basins (n=71)

Skeletal mineralogy; preservation, diagenesis and infilling

Preservation of original skeletal aragonite mineralogy is highly unusual due to the chemical instability of aragonite and its frequent alteration to secondary calcite. The combination of various methods has shown the exceptional mineralogical preservation and lack of taphonomic alteration of specimens from coral samples from both basins.

Using X-ray powder diffraction, over a 25° to 37° 2θ range, the majority of samples recorded seven main peak intensities. The main carbonate mineral peaks were four aragonite peaks (3.396Å, 3.273Å, 2.700Å, and 2.481Å) and one calcite peak (3.035Å). In addition to these, one quartz peak (3.343Å) and one gypsum peak (2.867Å) were recorded. Three samples show no peak for calcite (below 1% detection limit) and can be regarded as 100% aragonite with respect to their carbonate mineralogy.

Peak height analysis allowed the ratio for aragonite peak intensity to be determined. For *Acropora* specimens these ranged from 0.8299 ± 0.04 to 1 ± 0.05, with a maximum of 1. For *Lobopsammia* specimens these ranged from 0.9770 ± 0.05 to 1 ± 0.05. From this, the Milliman (1974) calibration curve allowed these values to be converted to percentage aragonite with respect to calcite. For *Acropora* specimens the percentage aragonite ranged from 92.55% ± 1.8 to 100%.± 2.2, and for the *Lobopsammia* specimens from 98.88% ± 2.1 to 100% ± 2.2.

The additional peaks seen in XRD across the 25° to 37° 2θ range, representing quartz and gypsum, are from infilling sediment or surface weathering (gypsum). In thin section and SEM, the quartz is seen infilling voids within the coral fabric together with minor components of clinopyroxene, biotite, glauconite and chert (Fig. 2e & f). The grains are subangular to subrounded and infilling corallites trapped particularly by intact septal structures.

All the *Acropora* specimens show some degree of cementation. The combination of thin sections, XRD, SEM and CL techniques indicates this as a minor component and confined to specific areas of the specimen. In thin section and CL there is evidence of both early aragonite cement and later calcite cement precipitation, infilling and lining porous areas. Bioerosion is minor with two of the *Lobopsammia* corals showing boring molluscs identified by their foliated internal structure.

In summary post-depositional modifications of the coral skeleton include entrapment of glauconitic fine grained sands and the precipitation of aragonite and calcite cements within the porous skeletal structure.

Stable Isotopes

For *Acropora* specimens the total variation is about 1.5‰ between -6.84‰ to -5.31‰ for $\delta^{18}O$ (Fig. 4). The range of $\delta^{13}C$ is between -4.17‰ to -1.63‰, showing a total variation of about 2.5‰. For *Lobopsammia* specimens the total variation is about 1.0‰ between -5.90‰ to -4.86‰ for $\delta^{18}O$ (Fig. 4a). The range of $\delta^{13}C$ is between -3.92‰ to -0.79‰, showing a total variation of about 3.1‰.

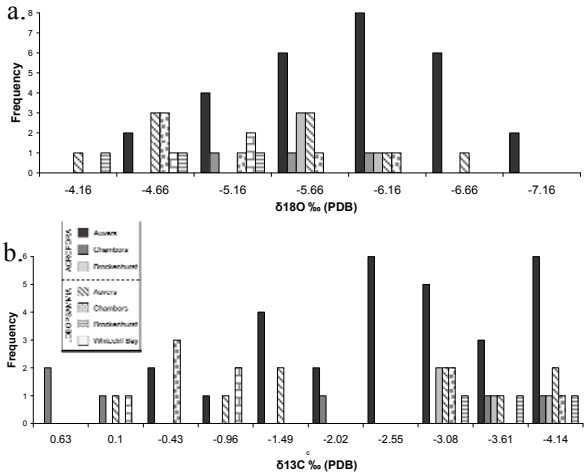

Figure 4: Frequency Histogram showing the distribution of a) oxygen and b) carbon isotope ratios for each locality of both Acropora and Lobopsammia specimens (n=60)

Carbon shows the larger variability in isotopic values, probably a function of the multiple factors which can effect this value (e.g. Zachos, 2001) (Fig. 4b). The *Acropora* specimens show a wide distribution with a skew towards isotopically lighter values. The *Lobopsammia* specimens show a weaker trend with a similar wide distribution of isotopic

values over a comparable range to *Acropora* specimens.

Discussion

Acropora is presently found within the modern reef coral limits, in all three major oceans of the world. The genus exists in a wide range of depths, reef zones and hydraulic regimes, primarily reflecting its ability for rapid growth. The fossil record shows that *Acropora* existed up to ~49 degrees north during the Eocene in the Hampshire and Paris basins. It is envisaged that in both basins *Acropora* occurred within isolated thickets/colonies, possibly forming more laterally extensive veneers, such as those seen in the marginal settings of Brownard County, Florida (Perry and Larcombe 2003). The relatively sparse distribution of specimens, and preservation as broken fragments in fine grained clastic sediments indicates that active reef accretion or mass reefal build-ups were unlikely to have occurred in either basin. If these specimens were derived from a reefal structure then unusually high hydraulic conditions would be needed to demolish evidence of this structure but to selectively preserve a limited number of pristine specimens. Additionally, the dominance of zooxanthellate and solitary corals, in modern settings typifies marginal environments, and the lack of other reef-building biota supports the absence of any reef.

The palaeoenvironmental settings are different within the two basins. In the Hampshire Basin, corals are found within siliciclastic storm beds in a relatively protected mid-shelf environment, below-wave base. Corals in the Paris Basin are found within predominantly carbonate-dominated beds and a mid-ramp environment is inferred. *Acropora's* ramose, branching habit renders the skeletal remains subject to fragmentation, through bioerosion, hydraulic energy, and rolling and abrasion by transportation. However, the preservation of these fragments, up to 4cm in length, with intact morphological features, supports a lack of transportation and reworking of the fossils. The lack of abrasion features shown by specimens from both basins indicates minimal transportation implying low hydraulic energy, protection from bioerosion and additionally rapid burial. Morphologically the sturdier growth forms come from the Hampshire Basin implicating higher wave energy in this basin relative to the Paris Basin

The pristine morphological and mineralogical preservation, and lack of bioerosion, of the specimens from both basins supports rapid burial following fragmentation of the coral branches. This infers high sedimentation rates. Rapid rates of sedimentation were required to produce a geochemically enclosed system and sufficient burial to prevent bioerosion by benthic organisms. Post-depositional modifications of the coral skeleton included entrapment of glauconitic sands and the precipitation of early aragonite and later calcite cement within the porous skeletal structure.

On the basis of modern distribution of zooxanthellate corals, the presence of *Acropora* within the two basins suggests tropical conditions persisted in Northern Europe during the Middle to early Late Eocene. This is supported by other palaeoclimatic indicators such as palaeobotanical studies (Collinson and Cleal 2001). This is confirmed by oxygen stable isotope values on specimens from basins indicating sub-tropical to tropical palaeoenvironments and the higher variation in carbon values reflecting the paralic environment in both basins.

The absence of reef rock and the existence of thickets and possible coral carpets suggests there was a lack of coral framework and raises the question as to whether this was a flourishing coral community or a disturbed/restricted state of development. The distribution of *Acropora* in the Hampshire and Paris basins during the Eocene is believed to have been at its northern limit. Combined with evidence that *Acropora* left these basins in the Late Eocene, existed for a geologically short period in each and that subsequently its northern limits retracted southwards suggests that this was a marginal setting. The lack of reefal build-ups however may have been due to evolutionary control rather than an ecological one as *Acropora* has not been found dominating reef frameworks until the Oligocene in Greece (Wallace and Rosen 2006).

In summary, the primary control on *Acropora*, and other coral genera, spatial and temporal distribution was primarily influenced by global and regional eustatic sea-level fall, climatic deterioration and local tectonics isolating both basins. Secondary controls included sediment supply and water movement. The existence of *Acropora* in the two basins reflects global climatic warm periods of the Eocene, its distribution reflects a complex interplay of local and regional factors. Global climatic and regional tectonic effects resulted in the loss of *Acropora*, and other corals, from the two basins by the end of the Late Eocene.

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Session number 1

Enigmatic Coral Rock Pillars – Another Look into Reef Dynamics

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Abstract. Stone capped calcareous pillars, rising 10-70 cm above the surrounding reef surface are to be found at Aqaba (Jordan) and on several other fringing reefs of the Northern Red Sea from intertidal down to 3 m depth. Terrigenous (non-calcareous) cobbles and boulders are fixed on top of coral limestone. At Aqaba it is assumed that the stones were once introduced by fishermen and came to rest in depressions of the reef. Afterwards the surrounding reef limestone was eroded so that only the substrate underlying the granite stones has been left as singular towers. ^{14}C-dating of a column sample provided an age of 345-560 years. Several interpretations are possible: the respective reef part did not grow since then, or younger layers were removed by bioerosion. Some suggestions are provided based on erosion data of sea urchins (*Diadema setosum*) and fish, gathered at that site. Two more examples of partial reef decline are presented from a reefflat at the Sinai coast north of Dahab indicating uplift along the margin of the Gulf of Aqaba and from the bay Marsa Bareika at the southern tip of Sinai where big boulders are interpreted as ballast stones discharged in an antique harbour.

Key words: Long-term reef shaping, bioerosion, Gulf of Aqaba (Red Sea)

Introduction

Some reefs at Aqaba (Jordan, Northern Red Sea) are under observation since 1972 (Mergner and Schuhmacher, 1974). They exhibit contrasting aspects from thriving coral communities with up to 60% living cover to barren limestone rock. The latter one is cleared by grazing fish and sea-urchins, especially *Diadema setosum*. Stone capped limestone pillars which rise 10-70 cm above the surrounding reef surface caused us to take a closer look at these structures and their history.

Similar structures found in other parts of the Red Sea are also shown.

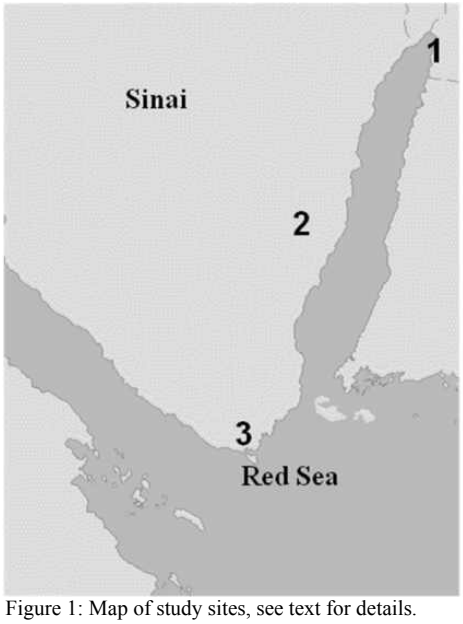

Figure 1: Map of study sites, see text for details.

Results and discussion

Findings from three sites of the Northern Red Sea are presented (Fig. 1):

1. Aqaba (northern end of the Gulf of Aqaba),
2. East coast of Sinai Peninsula between Dahab and Abu Galum,
3. Marsa Bareika, Ras Mohamed National Park, southern tip of Sinai.

In each case terrigenous cobbles and boulders are fixed on top of columnar elevations carved out from coral limestone.

Case study Aqaba:

Fig. 2 shows the top of a forereef mound at approx. 10 m depth off the Marine Science Station Aqaba. The arrow points to one of the cobblestones. The underlying pillar was identified as remnant of a *Porites* colony (Fig. 3). Its ^{14}C-dating revealed an age of 453 +/- 107 years. A neighbouring column made of a faviid skeleton was dated as to a maximum of 50 years.

It is assumed that the stones were once introduced by fishermen and came to rest in depressions of the reef. Afterwards, the surrounding reef limestone was eroded so that only the substrate underlying the granitic stones was left as singular towers. It is unknown when the stones were introduced, therefore several interpretations are possible: The respective reef part did not grow since decades/ages, or younger layers were removed by bioerosion, before the stone fell down. There is considerable bioerosion at that site: The density of *Diadema setosum* is 1.2 ind m^{-2}; the removal of carbonate substrate was calculated from gut contents and faeces analyses (considering

reworked material) as 1.023 kg m-²yr^{-1} (Kroll 1995, Reinicke and Schuhmacher 2008). Grazing fish, especially the acanthurid *Ctenochaetus striatus*, removed 1-3 mm yr^{-1} from *Favia* skeleton tiles which were exposed as colonization plates (v.Treeck et al. 1996 - regarding the impact of *C.striatus* see also Schuhmacher et al., session 10, this volume).

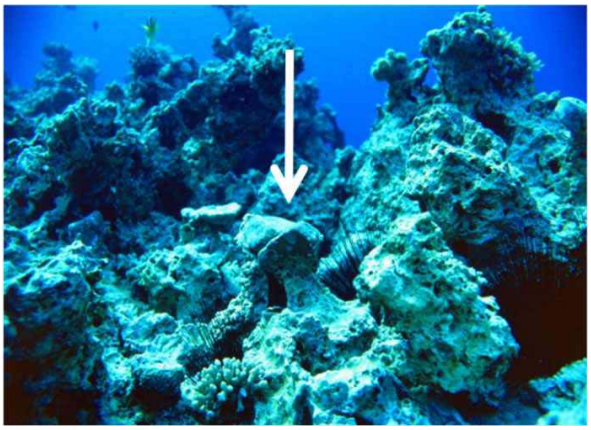

Figure 2: Granite stone on top of a forereef mound, Aqaba.

Figure 3: Stone capped *Porites* column; scale bar 7.5 cm.

Example case Sinai coast:

The mountains between Dahab and Ras Abu Galum steeply slope into the Gulf allowing development of only a narrow fringing reef. The reef flat continuously receives rubble tumbling down from the adjacent mountains. Fig. 4 shows the reef flat at low tide. At high tide the boulders are immersed except those on the tallest sockets. From the height of the calcareous columns it can be concluded that a 30-50cm thick layer was removed from the reef flat. The fact that the recent reef surface reaches to low tide level indicates a still considerable uplift of the western margin of the Gulf of Aqaba graben. Specific agents of bioerosion and time scales were not investigated.

Figure 4: Reef flat at low tide with terrigenous debris.

Example case Marsa Bareika:

Marsa Bareika is a large bay at the southern end of Sinai. A slightly inclined sandy wadi (river bed) enters the inner bay from the north. A poorly developed reef is interrupted by sandy areas. Several limestone outcrops, crowned by heavy boulders represent hardbottom islets that are sparsely colonized by corals (Fig. 5-6). The present topography does not provide an indication how the big boulders got to the site; anthropogenic transport, however, is likely. The bay is a natural harbour (today small vessels of the Ras Mohamed National Park are moored here). It is assumed that in ancient times (perhaps 2-4000 years ago) ships anchored at this site and dropped ballast stones – probably in exchange for copper or other minerals which were mined on Sinai and shipped to Egypt and further south. For a hypothetical bottom profile of that time see Fig.6. Archaeological studies have yet to be conducted. It would be intriguing to investigate ecological conditions and time periods, when this reef body formed and faded.

Figure 5: Boulder on top of a 70 cm high socket of reef rock.

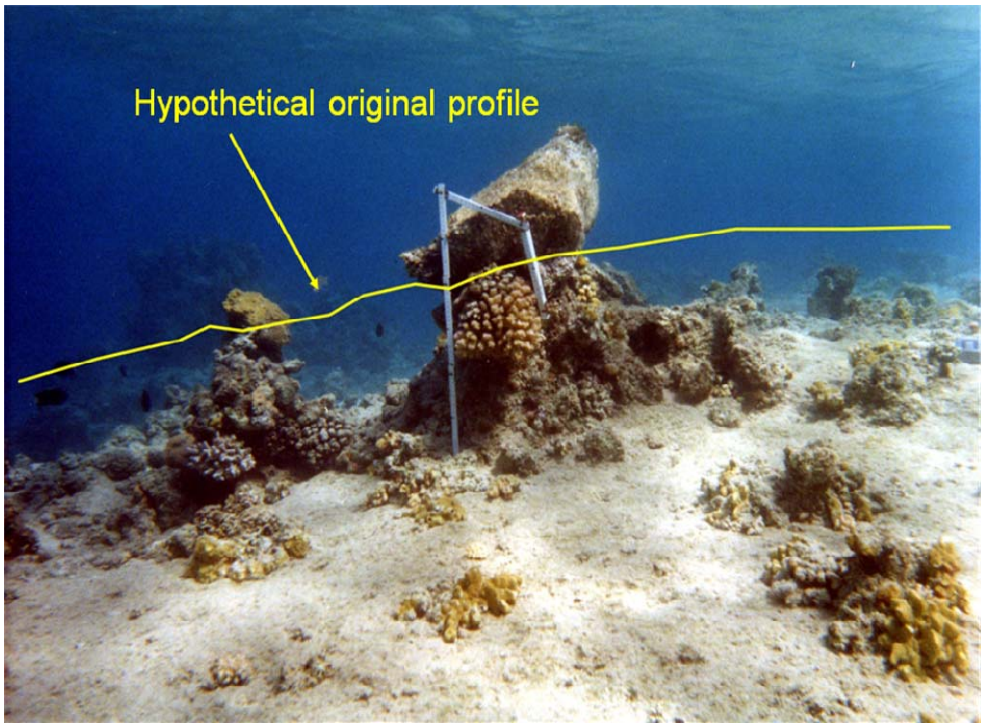

Figure 6: Boulder capped remnants of a former reef.

Conclusion

All three examples show reef areas, where the reef framework is eroding. The stone capped towers are remnants wittnessing of former reef developments. Anthropogenic reasons for the decline of these reef parts can be excluded. This presentation is thought to sensitize for this phenomenon at other places and to generate discussion about circumstances and modes of bioerosional reef shaping.

Acknowledgement

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Cenozoic Evolution of Larger Benthic Foraminifers: Paleoceanographic Evidence for Changing Habitats

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Abstract. The ever-increasing treasure-trove of paleoceanographic data relating to evolving Cenozoic ocean structure, including geographic and bathymetric gradients, provides novel insights into long-term changes in environmental conditions influencing shelf, ramp and oceanic-platform habitats occupied by carbonate-producing ecosystems. Similarly, recent studies documenting the influence of internal waves on mid- and deep-shelf habitats provides equally exciting insights into previously unrecognized environmental variability experienced by organisms living in those habitats. Paleocene-Eocene photic-dependent carbonates were dominated by calcitic coralline red algae and larger benthic foraminifers (LBF), with aragonitic corals and calcareous green algae more restricted temporally and spatially. Morphologies of LBF are strongly influenced by light availability and water motion, with larger, flatter and more fragile shapes characteristic of lower light, low wave-energy environments. Since substantial LBF habitat is at middle to outer shelf or ramp depths (i.e., ~30 to ~130 m), understanding the influence of internal waves on these habitats as oceanic thermal gradients developed through the Cenozoic can provide crucial insights into evolving environmental conditions where the most diverse and highly specialized LBF biotas occurred.

Key words: Carbonate ramp, reef, internal waves, thermocline gradients, symbiosis

Introduction

Understanding biological and geochemical processes associated with modern carbonate systems is essential to interpreting fossil reefs and carbonate sedimentation. Recognizing the limitations of uniformitarianism is equally crucial (Pomar & Hallock 2008). Cenozoic carbonate-producing ecosystems emerged from the remnants of Cretaceous biotas, evolving in the warm alkaline oceans of a Greenhouse world, then modifying as Icehouse conditions developed. The latter included stronger latitudinal and bathymetric temperature gradients, declining atmospheric CO_2 concentrations and declining calcium concentrations and alkalinity in the oceans.

Paleocene-Eocene photic-dependent carbonates were dominated by calcitic coralline red algae and larger benthic foraminifers (LBF), with aragonitic corals and calcareous green algae more restricted temporally and spatially. In this paper, we compare Hottinger's (1998) synthesis of records of diversifications and extinctions of Cenozoic LBF lineages in the context of published evidence for changes in ocean circulation and thermocline structure as Icehouse conditions developed (e.g., Lear et al. 2000). Moreover, given that much of the diversification was at middle to outer shelf depths (i.e., ~30 to ~130 m), synthesizing emerging records of the influence of internal waves at those depths (e.g., Wolanski et al. 2004; Leichter et al. 2005) with the records of oceanic thermal gradients through the Cenozoic (e.g., Dutton et al. 2005) can provide crucial insights into how environmental conditions changed for middle and outer shelf benthic habitats.

Larger Benthic Foraminifers (LBF)

The LBF are an informal group of benthic foraminifers characterized by relative large size (e.g., generally > 1mm and up to 6 cm in diameter – Lee 1998) and complex internal morphologies. Modern representatives host a rich variety of algal endosymbionts in a relationship similar to that between corals and their zooxanthellae. By analogy, fossil LBF are interpreted to also have hosted algal symbionts (e.g., Lee 1998; Hallock 1999; and references therein).

Extant LBF worldwide belong to members of six long-ranging families, the Amphisteginidae, Alveolinidae, Nummulitidae, Peneroplidae, Rotaliidae, and Soritidae (e.g., Hallock 1999). One other family, the Calcarinidae, is characteristic of the late Neogene in the Indo-west Pacific, where some species are prolific producers of beach sands (e.g., Hohenegger 2006). Amphisteginidae, Calcarinidae, Nummulitidae and Rotaliidae are all families in the order Rotaliida, and are characterized by relatively transparent (i.e.,

hyaline) calcite shells. Members of these families typically host diatom endosymbionts. In contrast, the other families are Miliolida, whose shells of randomly arranged calcite crystals covered by a veneer of brick-like crystals impart a porcelaneous appearance that is relatively opaque to light (e.g., Debenay et al. 2000). The shells of many of the larger miliolids have thinned outer walls to permit light into the chamberlets. Larger miliolids host a diversity of algal symbionts (e.g., Lee & Anderson 1991). The Alveolinidae host diatoms. One lineage of Peneroplidae hosts rhodophyte symbionts while a second lineage has chlorophyte symbionts. Similarly, one lineage in the Soritidae hosts dinoflagellate symbionts while the second hosts chlorophytes.

Distributions of modern taxa of LBF are strongly influenced by light (Fig.1). Both between species and within species trends in morphology, especially shape and surface-to-volume ratios, reflect light intensity and water motion (e.g., Hohenegger 2005), both of which influence rates of calcification (e.g., Hallock et al. 1986). Thus, both conceptual (Hallock & Glenn 1986; Beavington-Penney & Racey 2004) and numerical (e.g., Hallock 1987; Mateu-Vicens et al. 2008) models have been developed to interpret paleoenvironments of LBF-rich limestones.

Hallock (1988, 1999) also noted that some taxa, notably the porcelaneous Soritidae and the stellate Calcarinidae, have tended to diversify "laterally", specializing to different relatively shallow-water, higher light habitats, while modern Amphisteginidae, and Nummulitidae have diversified vertically, with robust shapes characteristic of shallower dwelling species and flat shapes characteristic of deeper depths.

Historically, the terms photic or euphotic have been used to describe environments where there was sufficient light for an excess of photosynthesis over respiration (e.g., Hallock & Schlager 1986). Pomar (2001) proposed terminology to distinguish among photic habitats. He used "euphotic" only for depths where light is sufficient for high rates of photosynthesis and associated hypercalcification, typically less than 30 m even in very clear water. He used "mesophotic" to characterize depths where light is sufficient for photosynthesis to support substantial calcification rates, but not true hypercalcification, i.e., depths in the 20-70 m range, again dependent upon water transparency. He used the term "oligophotic" to characterize depths where there is limited light penetration sufficient to support calcifiers like coralline red algae, and very thin flat LBF and corals. As Hallock (1987) demonstrated using a modeling approach, well developed biotas adapted to low light for photosynthesis depend upon consistently high water transparency that permits light penetration to depths >70 m. Such consistently high water transparency only occurs where surface waters are sediment free and have extremely low nutrient and plankton concentrations.

LBF assemblages typically characterize Pomar's (2001) light-defined zones (Fig. 1). Most of the miliolid LBF, as well as most calcarinids, live primarily in euphotic habitats. On the Florida reef tract, Soritidae (especially the Archaiasinae) show depth zonation, but none are common below about 30 m. Worldwide, robust *Amphistegina* also are found abundantly at less than 30 m, with progressively thinner or smaller chambered morphologies dominating below 30 m. Nummulitids can be found at less than 30 m, but most dominate at mesophotic to oligophotic depths (>30m, see Hohenegger 2005). Several extant nummulite species, whose shapes are very thin and flat, have depth distributions that peak at 70 m or deeper.

Water motion is another important environmental parameter influencing LBF distributions. Both wave energy and light decrease exponentially with depth, and both influence shell morphologies similarly, which is one reason LBF are excellent paleodepth indicators. Lenticular shapes, thicker shell walls and, in the case of the calcarinids, stellate morphologies allow such foraminifers to thrive and hypercalcify in high light, high wave-energy environments such as reef flats of the Indo-Pacific.

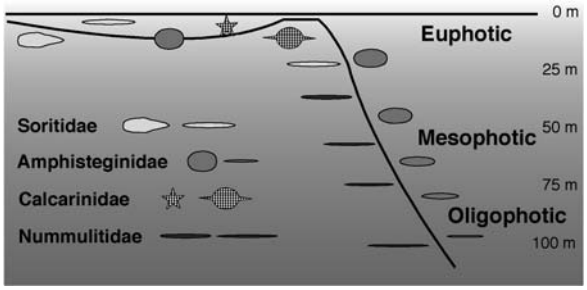

Figure 1: Major families of larger benthic foraminifers illustrating shapes and general depth ranges (Hallock 1999) in the context of euphotic, mesophotic and oligophotic habitats as defined by Pomar (2001).

In contrast, discoid morphologies are best adapted to quieter environments, where they either burrow their apertural face into the surficial sediments at a low angle, leaving most of the dorsal surface exposed to light, or sit flat on the substratum. Either way, they are superbly adapted to harvesting food and dissolved nutrients from the substratum and pore waters, while their algal symbionts capture radiant energy from sunlight. If their habitat is infrequently disturbed, e.g., by storm waves or deposit-feeding animals, these foraminifers can literally spend years accumulating the sparse resources required for reproduction (Hallock 1985). Exceptions to this generalization for discoid morphologies are shallow-dwelling Soritidae

that commonly attach to hard or phytal substrata in reef-flat environments.

Costs and benefits of algal symbiosis

There are energetic costs to establishing and maintaining symbioses between heterotrophic hosts and photoautotrophic symbionts (e.g., Hallock 1981; Stoecker 1998). Unless environmental conditions tip the energy balance in favor of benefits of symbiosis, photoautotrophic and heterotrophic taxa will predominate. The energy from photosynthesis must provide more than half the energy needed by the holobiont to be beneficial (Stoecker 1998).

Hallock (1981) predicted that, under nutrient-poor conditions with sufficient light, algal symbiosis can provide literally orders of magnitude energetic advantage over autotrophic-heterotrophic strategies. She further postulated that the most advantageous conditions for algal symbiosis occur when essential nutrients are extremely scarce and the only concentrated forms available are in particulate organic carbon such as plankton, bacteria or organic detritus.

Hallock (1985) examined life history strategies that would be most advantageous for LBF. She concluded that hydrodynamic environments, which tend to also be high light environments, would favor faster growth, higher fecundity and relatively short life spans. In contrast, Hallock (1985, 1987) predicted that, in mesophotic to oligophotic environments where radiant energy is limited but the probability of disturbance by hydrodynamic events is much reduced, longer life spans and production of fewer, larger embryons are advantageous. Mesophotic conditions appear to be optimal if water transparency is relatively dependable, because the holobionts are still able to get substantial energy from photosynthesis, while the potential for either photo-oxidative stress or physical damage by hydrodynamic events are low. The greatest risk is the potential for insufficient sunlight during turbidity events, although there is the potential for energy supplementation from feeding. Interestingly, the two extant families most prevalent in mesophotic and oligophotic depths are the amphisteginids and nummulitids. The former can become dormant within 24 hours of darkness, while at least some of the latter are bacteriovores (Lee 1998).

Oligophotic environments tend to be low energy, both radiant and hydrodynamic. So the limiting factor must be the tradeoff between the energy required to support the symbiosis versus the energy gain from photosynthesis. The morphological investment in maintaining a symbiosis appears to be extreme in very low light environments – long lives spent producing extremely large, flat shells. The strategies appear similar for zooxanthellate corals in oligophotic environments: significant investment in structures that optimize light capture rather than food capture (e.g., Jarrett et al. 2005). As a consequence, light energy must be relatively predictable for the investment to be beneficial, requiring that water column transparency be consistently very high.

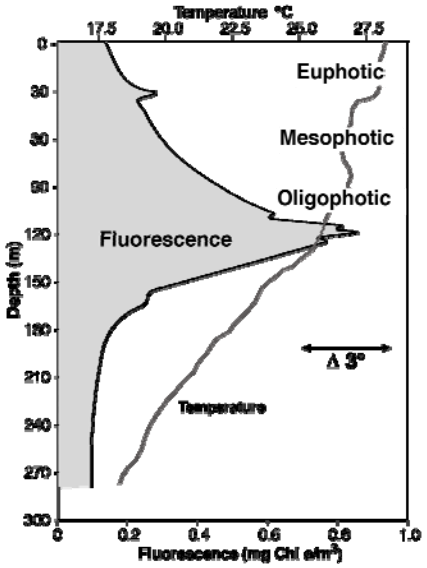

Figure 2: Data from Hallock et al. (1991) showing surface-to-thermocline temperature and fluorescence gradients for the modern northern Caribbean, annotated to indicate changes in light and temperature in the upper 150 m.

Bathymetric gradients and internal waves

In modern subtropical and tropical oceans, outside of upwelling zones, surface waters are warm and generally relatively well mixed by winds down to roughly 100 m. The base of the mixed layer/top of the thermocline also corresponds to the top of the pycnocline and nutricline, as well as the chlorophyll maximum zone (Fig. 2). In addition, O_2 concentration declines and pCO_2 increases relatively rapidly with depth. Above this layer, there is sufficient light for photosynthesis but generally limited nutrients. Below there is minimal light, but more organic carbon and biological activity utilizing oxygen, releasing nutrients and CO_2. This zone is very biologically and chemically dynamic.

The base of the mixed layer and uppermost thermocline corresponds with deeper mesophotic to oligophotic conditions described for outer shelf and ramp biotas. Thus, changes in physical and chemical conditions along that gradient would strongly influence deeper dwelling LBF biotas. The final consideration here is the influence of internal waves on LBF in mesophotic and oligophotic environments.

Leichter and colleagues studied internal waves in Jamaica and the Florida Keys, recording substantial temperature fluctuations on tidal frequencies at depths below about 30 m (e.g., Leichter et al. 2005; Leichter

& Genovese 2006). Wolanski et al. (2004) recorded temperature variations with depth in Palau, where strong internal waves impart as much as five degrees of temperature variability on habitats between 30 and 100 m depth on tidal cycles (Fig.3).

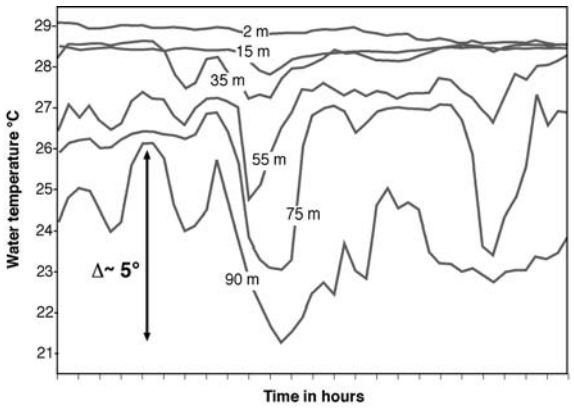

Figure 3: Data from Wolanski et al. (2004) recording internal wave activity on tidal cycles on the shelf of Palau, Western Caroline Islands, annotated to show that benthic communities living at 90 m depth experience substantial temperature fluctuations.

As a consequence, subtropical/tropical mesophotic and oligophotic biotas can experience temperature ranges of several degrees on internal-wave cycles. Those same organisms are experiencing comparable or greater changes in nutrients, organic carbon, and pCO_2. For LBF and other calcifying organisms, the latter may be particularly important as higher pCO_2 in the cooler waters, when pushed into shallower depths, should result in strong saturation gradients.

Cenozoic LBF: A brief summary

The end-Cretaceous extinction event (~65 Ma) was followed by an extended carbonate depositional hiatus (e.g., Newell 1982). Surviving shallow-water benthic foraminifers with established algal symbioses (e.g., miliolids) diversified, producing new lineages. New symbiotic associations developed in other lineages, especially diatom associations with rotalid taxa (Fig. 4). By ~60 Ma, new benthic taxa with complex morphologies were appearing (Hottinger 1998) and by the early Eocene, large, complex nummulitids were prominent carbonate-sediment producers in a variety of shelf and ramp environments (Beavington-Penney & Racey 2004). Extinctions eliminated the largest and most prominent forms of nummulitids and orthophragminids by the end of the Eocene.

New taxa arose in the Oligocene, the most prominently the lepidocyclinids and miogypsinids, though diversities, both horizontal and vertical were somewhat lower than during the Eocene apex (Beavington-Penney & Racey 2004). Lepidocyclinids and other characteristic Oligo-Miocene taxa were gone by the Early Pliocene. The amphisteginids, which arose in the Paleocene-Eocene, have since been represented through the Cenozoic, as have the Soritidae, Peneroplidae and new lineages of the Nummulitidae. With the Neogene closure of the circumtropical seaway and the isolation of Atlantic biotas, the archaiasine lineage of the Soritidae diversified in the Western Atlantic, while the Calcarinidae diversified in the Indo-west Pacific.

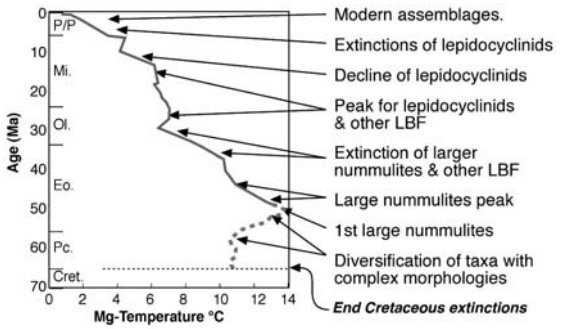

Figure 4: Comparison of the development of deep-sea temperatures (Leer et al. 2000) with diversification and extinction history of larger benthic foraminiferal (LBF) lineages (Hottinger 1998).

Given the prevalence of LBF as carbonate-sediment producers during the Cenozoic, especially during the Paleocene-Eocene, understanding and interpreting the environments in which LBF were living continues to be both scientifically and economically important. Hallock et al. (1991) compared Paleogene evolution and extinction events in LBF and planktic foraminifers, postulating that changing patterns in ocean circulation influenced nutrient regimes that were reflected in changing biotic assemblages. One goal of our paper is to consider how the evolution of oceanic conditions during the transition from the Greenhouse world of the early Paleogene to the Icehouse world of the Neogene influenced mesophotic to oligophotic habitats and biotas.

An interesting comparison can be made between the history of the LBF as interpreted by Hottinger (1998) and history of ocean stratification, as indicated by bathymetric temperature gradients that have been reconstructed for the subtropical north Pacific (Dutton et al. 2005). Note that the diversification of the large nummulites and other Paleogene LBF occurred when the deep sea was warmer (Fig. 4). The apex of these groups occurred when there was minimal temperature gradient between the thermocline and the deep sea, as reflected by minimal differences between $\Delta^{18}O$ in the shells of surface-dwelling planktic foraminifers and the shells of either *Subbotina*, which are thermocline-dwelling foraminifers, or shells of deep-sea benthic foraminifers (Fig. 5). In contrast, times when bottom temperatures were rapidly decreasing were characterized by increasing rates of extinctions of LBF (Fig. 4).

Directions for future studies

We postulate that the history of changes in Cenozoic ocean circulation associated with changes from early Paleogene Greenhouse to Neogene Icehouse conditions are recorded in evolutionary changes in LBF biotas. We recommend that future studies compare details of stratification of the ocean, as recorded by planktic foraminifers, with corresponding changes in LBF assemblages. We postulate that environmental conditions of middle and outer shelves can be more thoroughly interpreted by understanding changes in thermocline structure of impinging oceanic waters. Such progress will require more detailed comparisons of planktic and LBF records, and more research on the role of internal waves on modern outer shelf habitats.

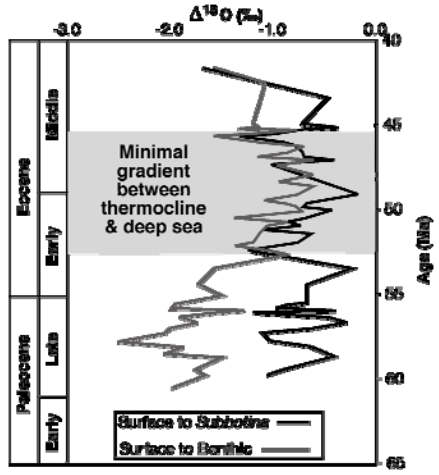

Figure 5: Data from Dutton et al. (2005) showing surface-to-thermocline (*Subbotina*) versus surface-to-bottom $\Delta^{18}O$ gradients, which indicate temperature gradients at the Shatzky Rise, subtropical North Pacific, during the Paleocene and Eocene. The bracketed time when differences between the gradients were minimal corresponds to the apex time for Eocene nummulites and other LBF according to Hottinger (1998).

Acknowledgement

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Environmental Factors Controlling Community Structure, Morphology and Linear Extension of Mid-Holocene Reef Corals from Cañada Honda, Southwestern, Dominican Republic

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Abstract. The Cañada Honda (CH) fossil coral reef, located in SW Dominican Republic, provides a unique opportunity to examine reef accretion in a high-sedimentation environment between 9.0-5.0 ky ago. Annual linear extension of fossil corals was determined for comparison with data from modern coral reefs. The reef is characterized by the high abundance of sediment-tolerant coral species, their tendency to form almost monospecific stands and grow as domes-cones with ragged margins. Also, colonies of *Montastraea faveolata* commonly contain bands of sediment incorporated within their skeletons. Calibrated radiocarbon ages of fossil corals range from 9,256±137 to 6,737±94.5 BP. Correlation with Holocene sea-level curves indicates that most corals on CH developed at depths greater than 15m. Growth rates varied from 0.09-0.44 cm/yr and suggest reduced light penetration caused by coral growth at such depths. Reef sediment is characterized by more than 85% carbonate material. A significant portion of the carbonate is allochtonous and was derived from nearby Neogene limestones. The reef was able to survive under these conditions because of the high carbonate content of incoming terrigenous sediment and that storms probably occurred sporadically providing intervening low-sedimentation periods during which reef corals could respond and grow back, "keeping-up" with sedimentation.

Key words: Holocene, siltation, linear extension, Caribbean

Introduction

Measurements of coral growth rates (as vertical extension) are used regularly as reef health indicators particularly in environments characterized by high terrigenous sediment input (e.g. Cortés and Risk 1985). Turbidity associated with suspended sediment and enhanced phytoplankton productivity reduces light penetration and the photosynthetic yield in corals (Cortés and Risk 1985; Philipp and Fabricius 2003) while the removal of settled particles from the coral increases metabolic costs (Telesnicki and Goldberg 1995; Fabricius 2005). As a result, a decrease in the annual growth rates of corals is expected.

For the past three decades a decline in reef coral abundance and diversity is being observed globally (Hughes 1994; Ginsburg 1994; Aronson et al. 2002) due to anthropogenic and natural causes. Increased coastal development and agriculture runoff leading to higher sediment accumulation and resuspension, together with discharge of sewage and industrial waste into the ocean have been blamed as major causes for modern coral reef decline (Hughes 1994; Hughes and Connell 1999; Morelock et al. 2001). A major question exists in terms of the relative roles that anthropogenic and natural disturbances play in the current trend of reef decline. The majority of the studies concerning reef health, siltation stress and low coral growth rates come from modern reef sites, many of them affected to a great extent by anthropogenic disturbance (e.g. Cortés and Risk 1985; Scoffin 1986; Edinger et al. 1998, 2000). Therefore it can be very difficult to differentiate the individual effects of natural versus anthropogenic disturbance. Some studies on coral reefs with natural high sediment input and resuspension show noteworthy differences compared with those with significant anthropogenic disturbance (Larcombe et al. 2001).

A reasonable way to differentiate anthropogenic disturbance versus natural disturbance is to study records of well-preserved fossil reefs in detail and compare the data with that gathered from modern coral reefs. This kind of approach has already been implemented in several studies (e.g. Pandolfi and Jackson 2001; Aronson et al. 1998; 2002), leading to the conclusions that current coral degradation has no precedent in the recent past. The purpose of this paper is to present data on fossil coral growth rates from the

Mid-Holocene Cañada Honda (CH) fossil coral reef of southwestern Dominican Republic (Fig. 1). The data presented is part of a larger study in which the main interest is to elucidate the pattern of reef development within natural conditions of high sedimentation and make comparisons with information gathered in modern reef environments under similar conditions. The CH fossil reef provides exceptional advantages that make it suitable for this kind of study. It displays remarkably well-preserved subaerial exposures of shallow-water reef environments dated between 10,000 to 4,500 years ago (Taylor et al. 1985). The young age (Mid Holocene) and the relatively dry climate has resulted in excellent preservation of original aragonitic skeletal mineralogy of fossil corals (Greer 2001). The CH fossil reef thrived in a high sediment environment as pointed out by Mann et al. (1984), Taylor et al. (1985) and Stemann and Johnson (1992) providing a unique opportunity for comparing a reef that developed under conditions of natural stress and disturbance with modern reefs that have been subjected to varying degrees of anthropogenic stress.

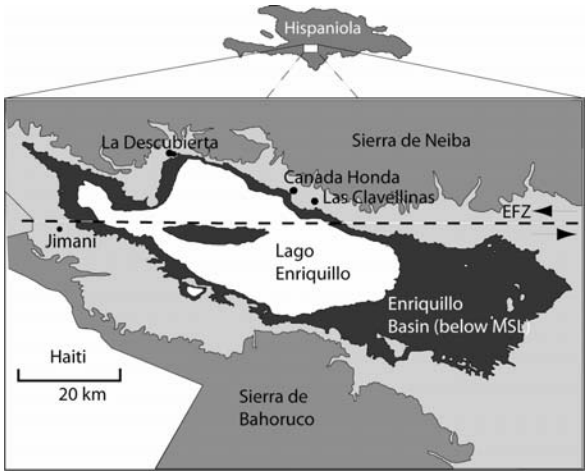

Figure 1: Map of the Enriquillo Lake area showing location of the Cañada Honda fossil reef. Black-colored areas represent land below present sea level. Dashed line represents location of the Enriquillo Fault Zone (EFZ).

Material and Methods

Field data collection

Variations in the sedimentary environments as well as coral assemblages were studied using quadrats along 18 vertical transects (Fig. 2) for a total of 67 quadrats. Information such as presence, abundance, morphology and distribution of corals were determined as well as the presence of other important taxa such as gastropods, serpulids, oysters and echinoderms. The importance or presence of reef sediment within the quadrat was also measured. Quadrats were placed on vertical exposures and each of the 100 points was counted as sediment or fossil. Locations for vertical transects were randomly selected and an effort was made not to bias placement on especially large or well preserved colonies.

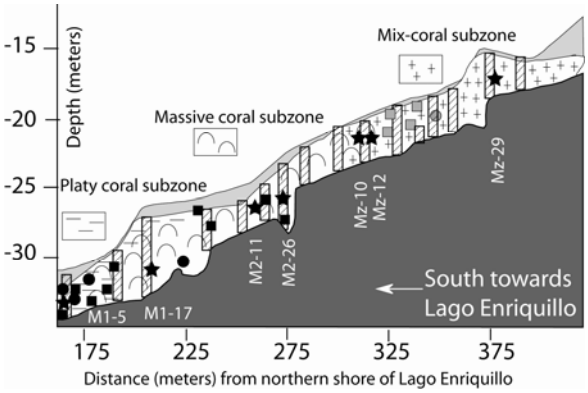

Figure 2: Cross-section of the Cañada Honda fossil reef showing location of transects (bars) and corals sampled for growth rate analyses. Squares represent *M. faveolata* samples and circles represent *S. siderea* samples. Difference in color denotes different subzones. Star represents samples for radiocarbon dates. *Acropora cervicornis* zone not shown.

Coral Growth Rates

Growth rate analyses focused on the corals *Montastraea faveolata* and *Siderastrea siderea* which are the two most abundant massive coral species in the CH fossil reef and additional growth rate data are available for each (e.g. Cortés and Risk 1985; Gischler and Oshmann 2005). Samples were collected randomly, identified and all the pertinent field information (photography, elevation, location in the reef) recorded (Fig. 2). To determine growth rates, corals were cut into 3-5 mm wide slices. The central slab of the colony, where the maximum growth occurs, was then polished and X-radiographed to reveal skeletal growth patterns. The radiographs were then scanned and the high and low density growth bands revealed by X-rays were measured using Coral XDS v. 3.0 (Coral X-radiograph Densitometry System) software that detects and measures changes in image density.

Radiocarbon dates

Seven of the coral specimens analyzed for growth rates were chosen for radiocarbon dating. The samples were selected according to their location on the CH reef in order to obtain coral ages at different elevations throughout the outcrop. In conjunction with well established Holocene sea-level curves (e.g., Toscano and Macintyre, 2003), radiocarbon ages were used to constrain paleodepths of the corals at their time of growth as well as determine the pattern of reef accretion through time. Radiocarbon analyses were conducted at the National Ocean Sciences AMS Facility of the Woods Hole Oceanographic Institution

in Massachusetts, USA. Radiocarbon ages obtained from the AMS facility were then calibrated to account for the ^{14}C local marine reservoir. Calibration was done using the CALIB software v. 5.1.

Sediment sampling and analyses

Nineteen sediment samples from the CH fossil reef were analyzed for this study. In addition, four sediment samples from a fluvio-deltaic sequence overlying the Mid-Holocene fossil coral reef from Las Clavellinas (Fig. 1) were analyzed for the purpose of determining composition of allochtonous terrigenous sediment that reached the former Enriquillo Bay. Analyses consisted of determination of the relative organic and carbonate content. A portion of each sample was weighed and then Clorox was added to remove organic matter. Samples were washed with distilled water, dried in an oven at 100˚C and weighed again to determine the percent of organic matter removed by the Clorox. This process was repeated again with a solution of 10% Hydrochloric Acid (HCl) to remove the $CaCO_3$ of the samples.

Results

Community structure

Twenty-two Scleractinian coral species were identified in the CH fossil reef (Table 1). The most abundant coral species is the massive coral *Siderastrea siderea* with approximately 47.4% of the coral counts followed by *Montastraea faveolata* (8.73%), *Undaria (Agaricia) agaricites* (7.66%), *Colpophyllia natans* (5.83%), *Montrastraea franksi* (4.13%), and *Acropora cervicornis* (3.86%). In general, the CH fossil reef can be divided into two major zones. The first, a branching or *Acropora cervicornis* zone which dominates throughout the upper reef (~0 to -15 m elev.). In this zone, the coral species *Acropora cervicornis* has an abundance of 37.9% followed by *Montastraea annularis* and *Undaria (Agaricia) tenuifolia*. The second zone or *Siderastrea siderea* zone consists mostly of massive corals where the coral species *Siderastrea siderea* range from 50% to 70% and can be divided into the following three subzones in descending elevation order (-15 to -35 m elev.); a high diversity assemblage or mix-coral subzone, a low diversity assemblage or massive coral subzone and a platy coral subzone (Fig. 2). A similar pattern of coral species distribution was obtained by Stemann and Johnson (1992).

Coral morphologies were grouped into five categories. These are platy, hemispherical, conical, columnar, and branching (Table 2). The dominant morphology in the CH fossil reef is hemispherical (34.9%), followed by conical (20.5%), branching (16.8%), platy (16.4%) and columnar(11.4%). Most of these morphologies are associated with reduced light penetration and turbidity caused by high sedimentation (James and Bourque 1992; Van Woesik and Done 1997). In addition, 51% of the counts from the massive coral morphologies (i.e. hemispheres, columns and cones) have ragged margins (Table 2) which is indicative of sedimentation rates equal or almost equal to the coral growth rates (James and Bourque 1992).

Coral Growth Rates

Twenty-five coral colonies were analyzed to determine annual growth rate. For the massive coral *Montastraea faveolata*, annual growth rates ranged from 0.09 to 0.44 cm/yr (Table 3). In coral colonies of *Siderastrea siderea*, annual growth rate varied from 0.2 to 0.4 cm/yr. Compared with *Montastraea faveolata*, *Siderastrea siderea* showed less variability in the annual growth rate values (Table 3).

Table 1. Distribution (% counts) of coral species and sediment in Cañada Honda

Coral species	C. Honda
Siderastrea siderea	47.38
Montastraea faveolata	8.73
Stephanocoenia intersepts	1.25
Madracis spp.	0.84
Dichocoenia stokesi	1.33
Colpophyllia natans	5.83
Agaricia lamarcki	2.05
Undaria agaricites	7.66
Acropora cervicornis	3.86
Oculina diffusa	0.41
Porites porites	3.86
Siderastrea radians	0.45
Helioceris cucullata	0.74
Mussa angulosa	0.14
Manicinia aerolata	0.23
Eusmilia fastigiana	3.18
Montastraea annularis	1.97
Montastraea franksi	4.13
Montastraea cavernosa	2.34
Porites astreoides	0.33
Undaria tenuifolia	1.46
Scolymia spp.	0.02
Other ‡	1.81
Sediment	22.62†

*out of 4871 coral counts

‡ Refers to unrecognizable, highly damaged coral colonies

†out of 6295 which is the total number of counts

Table 2. Summary of coral morphology distribution along Cañada Honda (% counts)

Morphology	Smooth	Ragged	Total %
Branching	16.8	-	16.8
Platy	16.4	-	16.4
Cones	10.5	10.0	20.5
Columns	8.6	2.8	11.4
Domes	13.7	21.2	34.9

Radiocarbon ages

Calibrated radiocarbon ages obtained from the seven CH coral samples ranged from 9,256.0±137 BP to

6,736.5±94.5 BP (Table 4). The oldest age is from a specimen of *Montastraea faveolata* (9,256.0±137 BP) followed by a specimen of *Siderastrea siderea* (8,628.0±165 BP). The youngest age was found in a specimen of *Montastraea faveolata* (6,736.5±94.5 BP) followed by a specimen of *Siderastrea siderea* (7,465.0±83 BP). Correlation of radiocarbon ages with a well-established Holocene sea-level curve (i.e. Toscano and Macintyre, 2003) helps establish the approximate paleodepth at which coral specimens grew. Except for coral sample Mz-29, which occurred in a topographic high (Fig. 2), the remaining corals developed at paleodepths of no less than 15m (Table 4).

Table 3. Results of measurements of coral growth rates from Cañada Honda

Coral sample	N	Mean annual extension (cm/yr)	Error (2σ)
Montastraea faveolata			
Pz-01	31	0.340	0.042
M1-5	28	0.091	0.013
M1-10	35	0.280	0.037
M1-13	31	0.248	0.019
M1-30	27	0.286	0.004
M2-6	22	0.363	0.024
M2-10	31	0.175	0.017
M2-11	107	0.142	0.014
M2-17	19	0.313	0.045
M2-21	12	0.278	0.062
M3-8	35	0.189	0.032
M3-18	21	0.299	0.055
M3-20	16	0.213	0.053
Mz-10	8	0.351	0.079
Mz-14	15	0.305	0.049
Mz-16	15	0.410	0.035
Mz-29	17	0.437	0.042
Siderastrea siderea			
M1-17	15	0.382	0.042
M1-22	17	0.269	0.033
M2-1	42	0.256	0.020
M2-2	8	0.400	0.092
M2-26	16	0.304	0.036
Mz-12	43	0.335	0.024
Mz-21	9	0.246	0.034
Pz-02	24	0.222	0.031

Sediment composition

Sediment composition analyses conducted on the nineteen sediment samples from the CH fossil reef are characterized by a high percentage of carbonate (i.e. $CaCO_3$) material (Table 5). Carbonate content varied from 84% to 96%. In contrast, the sediment samples from the fluvio-deltaic deposits in Las Clavellinas are characterized by carbonate content varying from 41% to 69% (Table 5).

Discussion and Conclusions

The Mid-Holocene Cañada Honda fossil reef in the Dominican Republic shows signs of development in an environment of high sedimentation based on the relatively high abundance of sediment-tolerant coral species, the tendency of these to form almost monospecific stands, and the propensity of individual colonies to grow as encrusting, dome-shaped, platy-like forms. Also the finding of sediment incorporated into coral skeletons supports the idea of siltation stress during the accretion of the reef.

Coral growth rates measured for fossil *Montastraea faveolata* in CH are relatively low compared with growth rates of modern corals from sites throughout the Caribbean. These low growth rates resulted from low light penetration most likely from growth at 15m or more (Table 4). Nonetheless, if these conditions are correct, coral development occurred at depths not expected for a reef under siltation stress. However, the high carbonate content of the incoming terrigenous sediment allowed greater light penetration than similar amounts of siliciclastic sediment, and,

Table 4. Summary of calibrated radiocarbon ages from coral samples in Cañada Honda

Coral sample	Cal. ^{14}C ages (BP)	Error (2σ)	Paleodepth (±1m)
Mont. faveolata			
M1-5	9,256.0	137	16.0 m
M2-11	7,882.5	99.5	18.0 m
Mz-10	6,736.5	94.5	16.0 m
Mz-29	7,705.0	106	8.0 m
Sid. siderea			
M1-17	8,628.0	165	18.0 m
M2-26	7,846.0	97	16.0 m
Mz-12	7,465.0	83	15.0 m

Table 5. Results of sediment composition analyses from Cañada Honda reef sediment and Las Clavellinas fluvial sediment (LCS samples)

Sediment sample	% Organic Matter	% $CaCO_3$	% Non-Carbonate
LCS-1	3	69	28
LCS-2	2	50	48
LCS-3	5	41	54
LCS-4	8	54	38
S-43-1	2	89	9
S-43-2	4	88	8
S-43-3	4	92	4
S-67-1	4	92	4
S-67-2	0	96	4
S-67-3	5	86	9
S-104-1	0	93	7
S-104-2	3	94	3
S-104-3	12	84	4
S-104-4	5	88	7
S-136-1	3	94	3
S-136-2	2	90	8
S-176-1	8	90	2
S-176-2	3	94	3
S-176-3	7	90	3
S-232-1	2	94	4
S-232-2	3	96	1
S-232-3	4	95	1
S-400-1	2	95	3

thus, allowed corals to grow at greater depths than typically expected under these conditions. Other possibilities are that even though sedimentation was high, it occurred sporadically allowing time for the reef corals to respond and grow back, in such a way that they were able to "keep-up" with sedimentation and sea-level rise.

The coral growth rates measured for *Siderastrea siderea* in CH are similar to growth rates of this coral from other sites in the Caribbean including sites in shallow water, with siltation stress and great depths. Also, *S. siderea* colonies from CH did not show clear bands of incorporated sediment into the skeleton compared with those of *Montastraea faveolata*. This fact highlights the ability of this coral to reject sediment and survive in stressful environments. Therefore, coral growth rates of *Siderastrea siderea* should not be taken as an unequivocal indicator of turbidity or other signs of reduced light penetration until more is known about the feeding mechanism, metabolism and skeletogenesis of this coral species.

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Results of long-term bioerosion study: Belize patch reefs

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Abstract. Bioerosion rates were tracked for up to 8 years on patch reefs in central Belize using natural and experimental substrates. Mortality from bleaching in 1998 provided the start of a natural experiment in which *Acropora cervicornis* and *Agaricia tenuifolia* were collected at 1-2 year intervals for 8 years. In addition, experimental substrates (conch shells) deployed along a depth gradient (1-12m) were retrieved at 1, 3, and 5 year intervals. Macroboring bioerosion rates were initially highly linear (5-6%/yr), and inversely related to depth. After 3-4 years macroboring bioerosion slowed and average maximum intensity tended toward approximately 40%. Individual substrates had a threshold of around 50% which may reflect the maximum gallery density for *Cliona* or the collapse and disappearance of more highly bored substrates. Wide spread coralline algae encrustation and the persistence of corallite surface texture suggests that external bioerosion from grazing was negligible. These results differ from previous studies that have found grazing bioerosion to be an order of magnitude or more greater than macroboring. This study highlights the greater importance of boring than grazing in several Caribbean reef habitats and the need to re-evaluate the relative impacts of inside-out vs outside-in bioerosion on coral reefs.

Key words: Belize, Bioerosion, Bleaching, Boring, Grazing

Introduction

In order to understand coral reef accretion, it is important to keep in mind that to a first approximation most biological coral growth is ultimately lost from the reef structure via bioerosion and transport (Glynn 1997). This is evident on apparently healthy reefs that have little accretion over geologic time (Smithers et al. 2006), in reef cores where fast growing corals do not result in faster reef accretion than slower growing corals (Gischler 2008), and in nutrient rich areas where bioerosion predominates over fast coral growth (Edinger et al. 2000).

Despite the importance of bioerosion to reef formation, there are few quantitative studies of bioerosion, especially in the Caribbean. Studies on bioerosion rates in the Pacific and Indian Oceans suggest that in most cases external bioerosion from scraping herbivores such as parrotfish and sea urchins far exceeds internal bioerosion by macroborers such as sponges and bivalves generally by more than an order of magnitude (Table 1).

The emerging outside-in, rather than inside-out paradigm for reef bioerosion is an important consideration for geologists who focus primarily on macroboring and microboring rather than grazing bioerosion (e.g., Perry and Hepburn 2008), and only recently have attempts been made to reconstruct grazing in the rock record (Lescinsky 2008).

The outside-in paradigm for reef bioerosion, however, is derived from studies that used sawed blocks of *Porites* that were bolted to the reef (Kiene and Hutchings 1994; Chazottes et al. 2002; Osorno et al. 2005). Results from these studies may be most applicable to flat, exposed reef-rock surfaces and they may be less applicable to other types of reef substrate and environments. Previously reported low macroboring rates are also at apparent odds with studies on experimental substrates (Neumann 1966, Schonberg and Wilkinson 2001) and naturally bleached corals (Sheppard et al. 2002; Schuhmacher et al. 2005) that document rapid sponge bioerosion. The grazing paradigm also does not hold for all reefs. Boring outpaced grazing at nearshore sites on the Great Barrier Reef (Table 1), and on the Belize patch reefs reported here.

Methods

Natural Experiment

Coral bleaching in 1998 killed healthy *Acropora cervicornis* and *Agaricia tenuifolia* stands on Wee Wee Cay (16.620 N x 88.090W) and Peter Douglas Cay (16.700 N x 88.170W) in southern Belize approximately nine miles off the east coast of the mainland in the Stann Creek District (Aronson et al. 2000, Paul Shave, pers. com.). Beginning two years after bleaching, coral pieces were haphazardly collected on snorkel from dead stands

along the north ridge of each cay. Collections were made after 2, 3, 4, 6, and 8 years (November or December of 2000, 2001, 2002, 2004, 2006). *Agaricia* patches were located just below the lip of the windward rise (3-4m) while *Acropora* dominated on the ridge tops (2-3m). Corals stands were obvious at the beginning of the study with many long (>20cm) pieces of *Acropora* and in-place *Agaricia* with many blades. By the end of the study, the colonies were largely obscured, especially because of coral resurgence on Wee Wee Cay beginning in 2006. Coral pieces retrieved at the end were smaller and many were detached rubble.

Experimental deployments: Conchs

Conch shells (*Strombus gigas*) harvested live for food and without macroboring were deployed along a depth gradient at 1.5m, 6m and 12m along the north face of Wee Wee patch reef (approximately 100 yards west of Wee Wee Cay). Only shells with a mature flared lip, and without encrustation (or with minor encrustation removed) were used. The 12m site was near the base of the slope above a flat plain of carbonate mud. Some shells were partially buried by fine sediment during the course of the study. Thirty or more shells were deployed at each of the three depth stations to allow collection of 10 shells each after 1 year (2002), 3 years (2004) and 5 years (2006). Shells were then sawed to remove the center section of the mature apertural lip for analysis.

Bioerosion Analysis

For corals, 20 pieces per sample (80 total: 2 species at 2 sites), per time interval were sectioned using a rock saw. For conch lips, 10 pieces per sample (30 total, 10 at 3 depths), per time interval were sectioned. Each piece was cut into three sections which were analyzed for bioerosion. Each section was digitally photographed and overlaid by a grid of points. Bioerosion proportion was determined by the number of points located over borings divided by the total points (>100). Microboring was not examined in this study.

Table 1: Results of studies that calculated both internal and external bioerosion. External generally exceeds internal bioerosion by an order of magnitude except in nearshore Great Barrier Reef (GBR) settings.

Location	Duration (months)	Internal bioerosion (kg m^{-2} yr^{-1})	Internal:external bioerosion	Reference
French Polynesia	24	.02-.14	1: 2.5-86 (av. 16)	Pari et al. 1998
	60	.02-1.04	1: 1.4-74 (av. 18)	Pari et al. 2002
Reunion	12	.006-.05	1: 61-263, (av. 130)	Chazottes et al. 2002
Lizard Island	60-84	.06-.24	1: 5.8-10.3	Kiene and Hutchings 1994
GBR nearshore	36-48	.25-.39	1: .16-.55	Osorno et al. 2005, Tribollet
GBR offshore	36-48	.04-.13	1: 2.6-112 (av. 27)	and Golubic 2005
Galapagos	15	.6-2.4, (0.6%/yr)	1:9.5	Reaka-Kudla et al. 1996
Belize	36	(6%/yr)	<<1	This study

Results

Natural Experiment

Over the eight years of study, dead branching colonies of *Acropora* and *Agaricia* became increasingly fragmented and in some cases overgrown, although former stands remained easy to locate and sample. Coral surfaces were often partially encrusted by coralline algae (Fig. 1). On unencrusted surfaces, original corallite topography, such as the raised polyp rims in *Acropora* was generally maintained There was little to no evidence of scrape marks from grazing bioeroders.

Macroboring was almost entirely from several ichno-taxa of boring sponge (undifferentiated in the data). Worm and bivalve borings were rarely encountered. Over the first 4 years, average macroboring bioerosion of the coral rubble proceeded at about 6%/year in a highly linear fashion (Fig. 2-3, Table 2). Rates decreased towards the end of the study. Variation between the three sections per sample and between samples was substantial but did not obscure overall trends.

Between sites there was a small but significant (ANOVA <.001) difference with Wee Wee having slightly greater total bioerosion over most of the study, even though after the first 3 years the overall rates (slopes of best fit line) were similar.

Bioerosion rates between *Agaricia* and *Acropora* were similar (6%/yr). Higher absolute "bioerosion" in *Acropora* in Fig. 3 results from the inclusion of the initial porosity of the coral skeleton. Unbored *Acropora* skeleton has initial growth pores that occupy a varying amount of the cut surface depending on the age of the branch and the angle of the cut. We used 5% initial porosity (an average of several measurements) as our initial condition (y-intercept).

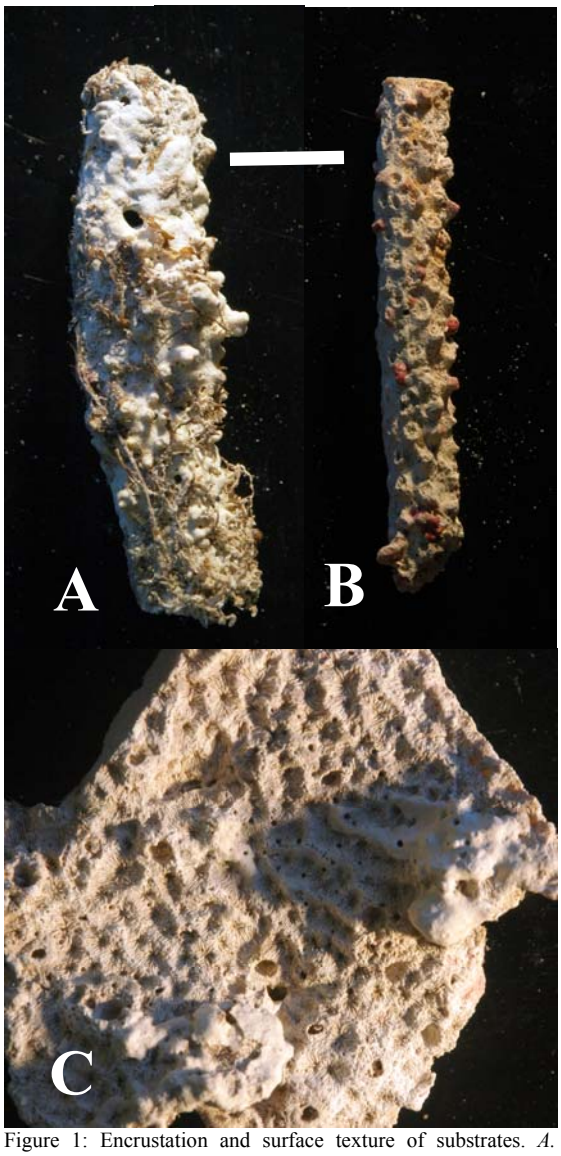

Figure 1: Encrustation and surface texture of substrates. *A. cervicornis*, 6 yrs (A) has knobby coralline algae and lacks evidence of grazing bioerosion. *A. cervicornis,* 8 yrs (B), partial retains corallite collars with limited encrustation by *Homotrema rubrum*. *A. tenuifolia*, 6 yrs (C), preserves original corallite topography. Scale bar is 1 cm.

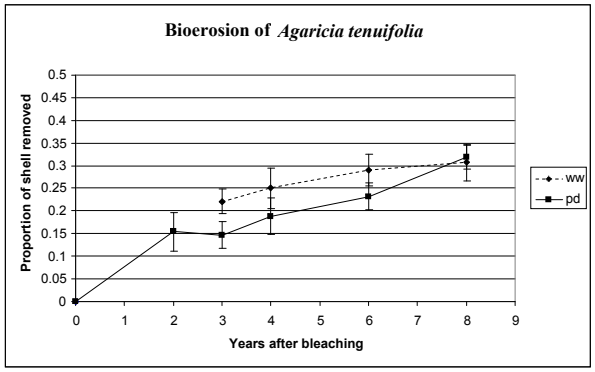

Figure 2: Bioerosion over 8 years for *Agaricia tenuifolia* killed in the 1998 bleaching. Mean values and 95% confidence intervals for Wee Wee Cay (ww) and Peter Douglas Cay (pd). Bioerosion has a nearly constant rate over the first 4 years of 5-6%/year. .

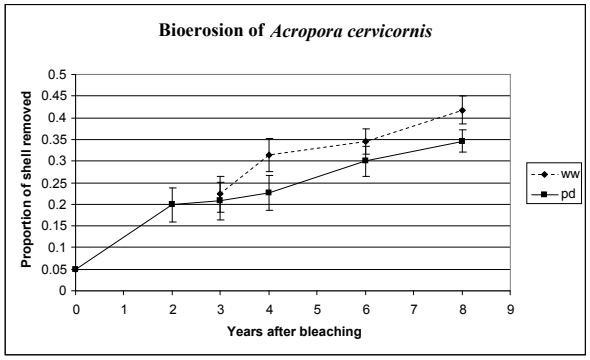

Figure 3: Bioerosion over 8 years for *Acropora cervicornis* killed in the 1998 bleaching. Mean values and 95% confidence intervals for Wee Wee Cay (ww) and Peter Douglas Cay (pd). *Acropora cervicornis* has a living porosity of 5-10% (depending on age, angle of cut); 5% is used here for the y-intercept.

Experimental Substrates

For the first 3 years, experimental conch shells were macro-bored at around 9%/yr at the two shallower depths, a rate higher than those of natural substrates at similar depth. There was no significant difference between bioerosion at the two shallower stations, but the deepest station (12m), bioerosion decreased to about a half (4.4%/yr).

Table 2: Bioerosion rates (proportion of skeleton removed per year) as measured as the slope of best fit line of the average of both sites. R^2 values for linear regression lines are high over 3+ years suggesting a tight correlation between time and macroboring intensity.

	Bioerosion Rate:	**R^2: 3 yrs**	**R^2: 4 yrs**
Agaricia	.063	.97	.95
Acropora	.058	.94	.96
Strombus (1.5m)	.096	.999	
Strombus (6m)	.092	.97	
Strombus (12m)	.044	.996	

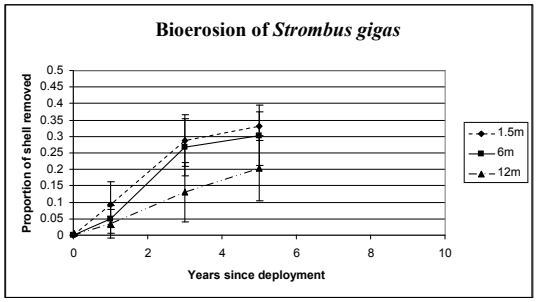

Figure 4: Bioerosion over 5 yeas for experimentally deployed *Strombus gigas* shells. Over the first 3 years the bioerosion rate is linear at about 9%/yr for <6m and 4.4%/yr for 12m sample. Mean values with 95% confidence intervals.

Discussion

Direct comparisons of the rates reported here (6-9%/yr in shallow water) with those from previous studies are difficult because bioerosion rates are generally reported as a function of weight loss per reef surface area (kg m^2yr^{-1}). This metric is easily applied to small flat experimental plates but is more difficult to apply to complex 3-D substrates or where the vertical extent of boring is not known.

In this study, bioerosion of 6% yr^{-1} at a density of about 1 g/cm^3 (Boucher et al. 1998) corresponds to a weight loss of 60kg m^2 yr^{-1} for a 1m cube and .6kg m^2 yr^{-1} for a $1m^2$ slab 1cm thick. To correct for surface area of the coral we can divide this number in half for tabular colonies with erosion on two sides (ex. *Agaricia*, *Strombus* shell) or for cylindrical colonies (e.g., *A. cervicornis*) a number depending on diameter (1cm: .25, 2cm: .5, 4cm: 1). To relate colony surface area to reef planar area, an additional multiplication factor would be needed since the former may be many times the latter.

Given the assumptions inherent in the conversion, we suggest that 6% yr^{-1} translates to around .3 kg m^2 yr^{-1}, and rates 4-5x greater are appropriate if related to planar reef surface. Within this range of rates, macroboring bioerosion on the Belize shelf is considerably higher than reported in most previous studies (Table 1), except where living sponges were attached directly to substrates (e.g., Neumann 1966, Rutzler 1975).

An important conclusion of the study is that bioerosion rates were surprisingly linear and similar between sites and substrate types (Fig. 5). For example, after 3-4 years, best fit lines had R^2 values .95 or greater for all three substrate types (*Agaricia*, *Acropora,* and *Strombus*). There was no time lag in the onset of macroboring has been suggested in some previous studies (Bromley et al. 1990).

Using linear bioerosion rates as a tool for estimating removal time for dead corals, or as a proxy for post-mortem exposure requires two important caveats. The first is that an upper limit to macroboring probably exists. Boring sponges have characteristic gallery patterns and may switch to lateral extension after excavating about 50% of the substrate (Rutzler 1975). Bored substrates also become increasingly fragile and will eventually collapse into smaller pieces that would be omitted from collections. For example after 5 years, several conch shells were missing parts and collapsed during retrieval.

A second consideration is that bioerosion is greatly slowed by burial. On reefs, vast differences in bioerosion and other taphonomic metrics occur between substrates resting on the seafloor, and those located above it on racks (Lescinsky et al. 2002). Intervals of burial and excavation may mix substrates of very different ages. For example, mollusc shells of similar preservation may be hundreds of years different in age in some settings (Meldahl et al. 1997).

The results of this study also call for a re-evaluation of the outside-in paradigm for reef bioerosion. Although previous studies (Table 1) have found grazing to be an order of magnitude greater than macroboring this is not the case for branching corals on Belize patch reefs. Grazing rates, although not measured directly are apparently minimal because coral surface texture is retained and there are thick coatings of coralline algae. In addition, if outside-in bioerosion rates were comparable to macroboring, our small substrates, would have disappeared entirely over the course of the study.

It is likely that we grazing bioerosion dominates macroboring bioerosion in many reef settings worldwide, though our results may also reflect in-part low grazing bioerosion in the Caribbean. As pointed out by Bellwood and Choat (1990) the Pacific has a diverse complement of excavating parrotfish but in the Atlantic *Scarus* species are all functional scrapers and only *Sparisoma viride* (stoplight parrotfish) is a significant bioeroder. In addition, bite size and bioerosion are related to fish size, and *S. viride* is dwarfed in size by the most important bioeroders in the Pacific, such as *Bolbometopon* which accounts for 88% of the bioerosion on outer reefs (Hoey and Bellwood 2008). Regional die-off of *Diadema* sea urchins would also lower grazing bioerosion in the Atlantic. Overfishing on the Belize reefs is probably not a direct cause because *S. viride* is abundant there (Rotjan and Lewis 2006, Brown-Saracino et al. 2007).

While regional processes may contribute to the differences described here, differences in methods were also likely important. The substrates in this study were small plates and branches of colonies that have numerous cryptic surfaces that would be difficult for large parrotfish to feed on. Similarly, invertebrate grazers such as sea-urchins may stay primarily on reef-rock and avoid branching corals and rubble (e.g., Bak

1990). Bioerosion of branching corals and corals with complex colony form, therefore, probably proceeds very differently than bioerosion of massive corals. When viewed from the perspective of the whole-reef, this is very important since branching corals are fast growing, dominate many shallow reef zones, and comprise most reef rubble. Macroboring probably also dominates in reef zones with low grazing, such as deeper water (Fox and Bellwood 2007), and throughout the reef in crypts which may comprise as much as 75% of the reef surface area and support diverse suspension feeding communities (Richter and Wunsch 1999).

In conclusion, bioerosion on the patch reefs of Belize occurs primarily through macroboring rather than grazing. This may in part result from differences of grazing bioeroders in the Caribbean, but it is also probable that inside-out bioerosion is a dominate force on most reefs in many zones. With the exception of large dead coral heads and smooth reef rock, boring may dominate in other situations such as where branching coral and rubble are abundant and in deeper water and crypts where grazing is greatly diminished.

Acknowledgements

We would like to thank P. and M Shave, directors of Wee Wee Cay Marine Lab, for logistical support and Otterbein College for research funding.

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Reef slope failure in the northeastern corner of Malè, Maldives

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Abstract. This study examines reef-slope failure that occurred on the northeastern corner of Malè Island on the 6th of February, 2002. Malè, the capital of the Maldives, has an area of 1.5 m^2 and a population of 130,000. The failed slope area was mapped and systematically investigated by comparing aerial photographs and satellite images from 1969 to 2007. The investigation shows that the reef-flat caprock consists of 2 to 3 m of slightly cemented coral and rubble overlying 4 to 6 m of weakly cemented, highly erodible coral sand and rubble located 10 to 30 m down the 30° slope. The failure may have occurred as a single event during which an integral rock mass disintegrated into individual blocks. Radiocarbon dating of the rock samples indicates rapid vertical reef growth, but horizontal progression played a more important role in reef formation. Aerial photographs and satellite images show four additional reef-failure areas on the northern side concentrated in areas of heavy urban-development overburden and continuous vibration from industrial activities. Due to reef failure, the strongly cemented caprock was removed, and the weakly cemented highly erodible layer was exposed to further erosion. The eroded parts are expected to fall into the atoll lagoon and cause further retreat of the reef edge. Proper remedial action must be taken to maintain overall stability and ground-bearing capacity of the island.

Keywords: Reef; slope failure; Malè; Maldives

Introduction

Malè is the capital city of the Maldives with an area of 1.5 m^2 and a population of 130,000 people. The island, located within and along the south eastern edge of a large north/south-trending atoll, has been reclaimed to the maximum extent possible, and heavy tetra-pod breakwaters have been constructed on the reef flat around the island. Heavy weight from new constructions, buildings, and industrial activity burdens the reclaimed area (Fig. 1).

Submarine reef slope failure triggered 19 days after a barge collision incident with an existing solid jetty that had been built on the reef flat in the northeastern corner of Malè in 1999. Two years after jetty completion and onset of industrial construction, the slope in that area failed in February 2002. Blocks of reef flat fell into the atoll lagoon, creating a number of new cracks that developed in various parts of the reef flat and slope. Some of these cracks extend 20 to 30 m into the slope.

Generally, slope failure was initiated by stress construction at the edge of the slope and was followed by cracking. Major factors that govern slope stability are slope angle and material properties, such as cohesion and the angle of friction. Typically, slopes with an angle of 20 to 30° and low cohesion are vulnerable to failure. In this case, however, the slope angle was around 10 to 15° and the material consisted of very cohesive and strongly consolidated 1- to 2-m thick reefal limestone. Thus, it is suspected that slope failure was affected by stresses induced by the excessive weight put on the island subsurface.

Study Methods

Investigation and systematic monitoring of collapsed reef area on the northeastern corner of Malè has been ongoing since 2004. The geologic structure of the collapsed area was mapped and systematically classified following general procedures in geotechnical engineering.

Cubes of collapsed blocks were measured and depths were recorded along three profiles that emanate from the edge of the reef flat and follow the reef slope onto the atoll lagoon floor to a depth of 45 to 50 m. The intra-reef structure was observed at the collapse scarp from the reef surface to –35 m. Rock samples collected from various depths of the collapsed area were dated using the radiocarbon method. To monitor the progress of cracks in the collapsed area, two pegs were installed on both sides of the crack and tightly connected with fishing lines. Aerial photographs and satellite images of Malè reef were taken in 2007 and were compared with the previous images taken in 1969, 1996, and 1999.

Results and Discussion

The reef-flat caprock consists of 2- to 3-m-thick slightly cemented coral rock and rubble. Beneath the caprock is a 4- to 6-m-thick weakly cemented highly erodible mixture of coral sand and coral rubble. Farther down (10-30 m), the 30° slope has been built up by loose gravel and sand.

Figure 1: A Digital Globe® satellite image of Malè taken in 2007, top, aerial photograph of north eastern corner of Malè taken in 1999 at the bottom, showing the solid jetty prior to the collapse.

The block field lies on the atoll lagoon floor at a depth of 30 to 40 m. The failure might have occurred as a single event, and as the rock mass was in motion, the mass disintegrated into individual blocks to form the block field. Total volume of the rockslide is estimated to be several thousand cubic meters (Figs. 2 & 3).

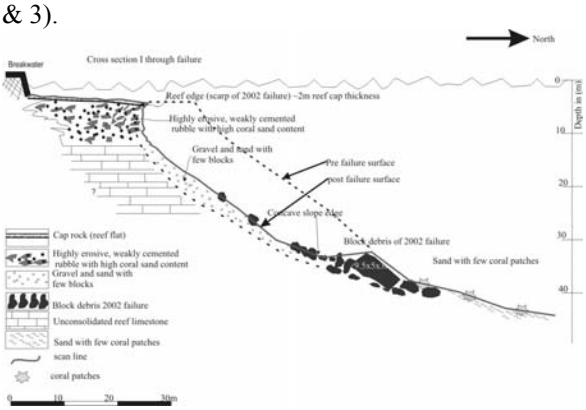

Figure 2: Cross section of failure area.

The monitoring pegs installed in 2004 have been broken. In addition, new cracks to the east of the collapsed area were observed in 2007. The cracked blocks appear to be unstable and continuously and slowly moving (ERC 2007).

Both the eastern and western scarps as well as the collapsed part of the reef are exposed to strong currents and wave action. Thus, the weak layer in the reef is continuously being eroded and is undercutting the reef edge. A freshly fallen reef block was found during the survey.

The collapsed scarp shows that the reef surface is consolidated at the top 2 to 3 m by the accretion of tabular, massive, and stubby branching corals. The sedimentary structure changes gradually to the coral casts near the bottom of the scarp (Fig. 4).

Radiocarbon dating of rock samples shows the oldest material occurs at the 20-m depth, and the youngest is at the top of the reef. This indicates that, even with the rapid growth of the vertical reef section, the horizontal progression of the reef played a more vital role in reef formation.

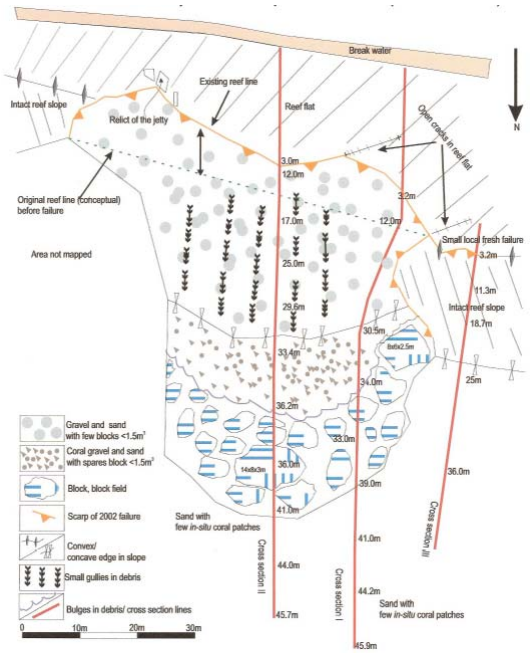

Figure 3: Plan view of failure area.

The reef surrounding Malè Island is continuous, and no collapsed area is found in aerial photographs taken in 1969 (Fig. 5). The reef area of the northeastern corner had not failed, whereas the area on the northwestern part had failed. Furthermore, satellite images taken in 2007 showed failure at four circled areas (Fig. 6). Those failures are concentrated in the areas of heavy construction and continuous industrial vibration. Although these areas are clearly visible in the photos, they need to be validated through field observations.

The study found that the geomechanical cause of the slide is a combined weakly cemented layer of coral sand and rubble several meters thick and urban construction. Similar subsurface conditions were determined by core drilling at Rasdhoo atoll (Gischler et al. 2008).

It is evident that the reef scarp, including the weak layer, is exposed to prevailing current and wave action and hence to active scouring beneath the consolidated layer. It is expected that parts of the reef will continue to fall into the atoll lagoon, leading to additional backward retreat of the reef edge toward the breakwater.

Further investigation is required to determine the lateral extent of the weak layer. Such an investigation is crucial prior to taking any remedial measure to stabilize the unstable reef slope. Further analysis of slope stability is also needed (Damjanac 1999).

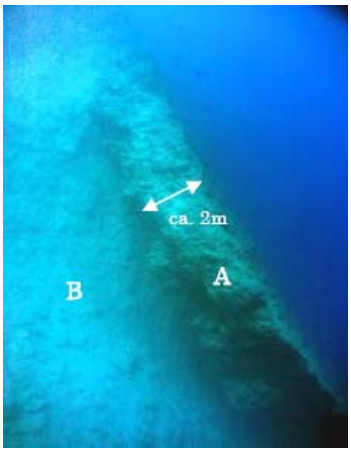

Figure 4: Scarp of the collapsed area showing the composition of the Malè house reef: A is the consolidated hard layer and B is the unconsolidated reef framework.

Figure 5: Aerial photograph taken in 1969.

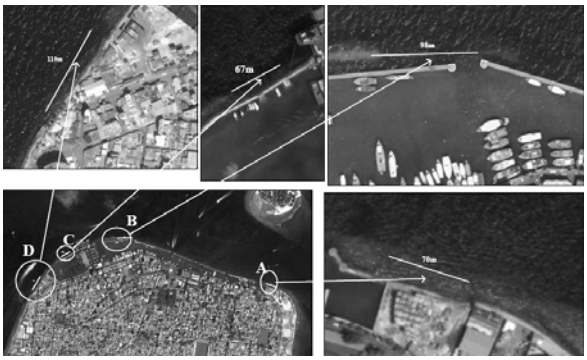

Figure 6: Magnified view of satellite image (2007) showing the failed areas on the northern reef of Malè.

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Real-time coral stress observations before, during, and after beach nourishment dredging offshore SE Florida

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Abstract. Beach nourishment in Southeast Florida involves dredging sand source borrow areas located between offshore reefs. From May 2005 to February 2006 Broward County, FL. nourished 10.9 km of beach with 1.5 $\times 10^6$ m^3 of sand. As part of a program to monitor potential reef community impacts, a visual stress index was developed from laboratory experiments and histological analyses for three stony coral species (*Montastrea cavernosa*, *Solenastrea bournoni*, and *Siderastrea siderea*). Scoring involved healthy = 0; moderately stressed = 1 (polyp swelling, increased mucus); markedly stressed = 2 (coloration changes, increased mucus secretion, tissue thinning); and severely stressed = 3 (severe swelling/thinning tissue erosion/necrosis). Colonies were scored weekly at sites adjacent to borrow areas and control sites pre-, during, and post-dredging. Permit conditions were established which would suspend dredging based on mean stress index values above 1.5 at 50% of monitored sites adjacent to borrow areas. This condition was never met. However, three hurricanes, passing the region during dredging, contributed to an elevated mean stress level above 1.0. Post-dredging observations documented recovery to pre-dredging stress levels. This program was effectively used to monitor stress on a sensitive marine habitat adjacent to sediment dredging activities.

Key words: Coral stress, dredging, beach nourishment.

Introduction

Beach nourishment in Southeast Florida is commonly accomplished by dredging from sand deposits located between offshore coral reefs and moving the sand to shore. Broward County, Florida, USA constructed 10.9 km of beach from May, 2005 through February, 2006, utilizing a hopper-dredge and placing 1.5 million cubic meters of sand, from 5 different sand borrow areas (Figure 1), onto the beach. Dredging can cause elevated turbidity due to suspension of sediments. This can result in stressful conditions, including smothering and/or reduction of photosynthesis, for sessile reef organisms, such as stony corals. To monitor a real-time response of stony corals to a potentially increased, sediment induced, stress environment during the dredging process, an observational stress index was developed for three coral species (*Montastraea cavernosa*, *Solenastrea bournoni*, and *Siderastrea siderea*). This stress index was incorporated into State and Federal permits issued for the dredge operation and an average stress index threshold was developed to allow for cessation of dredging at specific borrow areas should the stress threshold be exceeded. Recovery to below threshold stress levels would subsequently allow dredging activity at the specific borrow area to resume.

Material and Methods

The stress index was developed in the laboratory and verified histopathologically (Vargas-Angel, et al., 2006). Average values above 1.5 (n=6) at 50% of sites surrounding a particular borrow area would trigger cessation of dredging at that borrow until average stress fell below threshold. Figure 2 shows typical observable stress responses of *Montastraea cavernosa* for each stress index level. Treatment study sites were established adjacent to each borrow area (Figure 1) and control sites established 4-5 km north (3 sites) and south (5 sites) of the dredge operating area. Six colonies among the three species were selected at each site. Sites were visited and stress observations made weekly for thirteen weeks pre-construction, weekly during construction (38 continuous weeks with two exceptions due to tropical cyclone activity, and weekly for eight weeks post-construction. Additional observations from non-project colonies were made occasionally from outside the study sites to verify that visual observations were close or identical to histological stress determined in the lab.

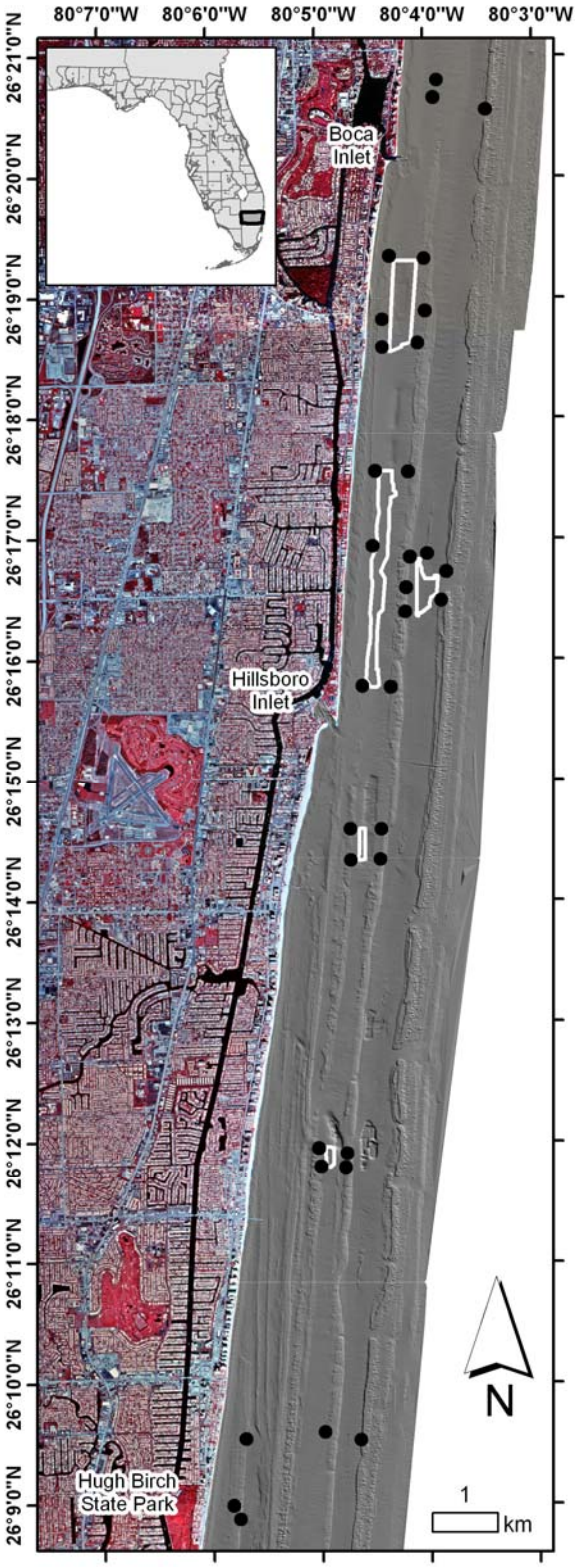

Figure 1: Map depicting sun-shaded laser bathymetry of the bottom offshore Broward County, Florida, USA. Reefs can be seen as ridges paralleling the shoreline (see Walker et al, 2008 for maps of the reefs). Dark circles are sediment monitoring sites where coral stress observations took place. Light colored boxes denote sand borrow areas between the reefs.

Figure 2a: STRESS LEVEL 0: Color appears normal; polyps are fully extended or loosely retracted; no tissue swelling; no apparent mucus sheets over colony.

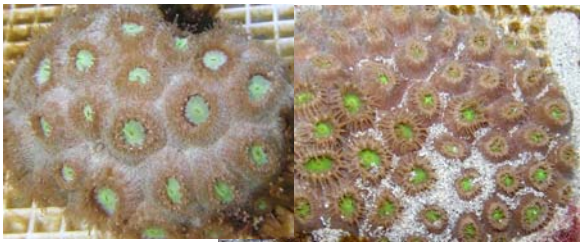

Figure 2b: STRESS LEVEL 1: Normal coloration, natural texture lines. Polyps are fully extended or loosely retracted; no apparent mucus sheets over colony; slight tissue swelling some unusual mouth opening shape and changes in the appearance of oral disk.

Figure 2c: STRESS LEVEL 2: Normal coloration to patchy bleaching. Normal texture lines begin to disappear; polyps appear "melted"; severe tissue swelling and apparent tissue thinning; presence of mucus sheets and sand accumulations between polyps.

Figure 2d: STRESS LEVEL 3: Coloration dull to brown to bleached; obvious tissue erosion and necrosis; polyps tightly retracted, deflated-looking with sunken-in oral disk areas; tissue thinning; skeleton starts to appear thru tissue. Mucus sheets present and colony shows an inability to remove sediments.

Results

Figure 3 presents photographic evidence that the observed level of stress for a *Montastraea cavernosa* colony from one of the treatment sites changed from level 0 to level 2 during construction and returned to level 1 after construction was completed. Average stress values (Figure 4) at treatment sites (n = 26 sites and 156 colonies) and control sites (n = 8 sites and 48

colonies) remained below 0.5 during the thirteen week pre-construction period and through the 14th week of dredging. The passing of Hurricane Katrina during construction week 15, Hurricane Rita during week 19, and Hurricane Wilma during week 24 contributed to immediate elevated observed average stress values between 0.5 and 1.0. Average histological stress was the highest during the summer maximum temperature (31.9^{o}) that occurred in August, 2005 (Vargas-Angel, personal communication). Average stress remained between 0.5 and 1.0 for the eight weeks of post-event analysis, indicating a recovery period to pre-impact levels longer than 8 weeks.

Figure 3a. Example of a monitored *M. cavernosa* colony at stress level 0 during construction on July 5, 2005.

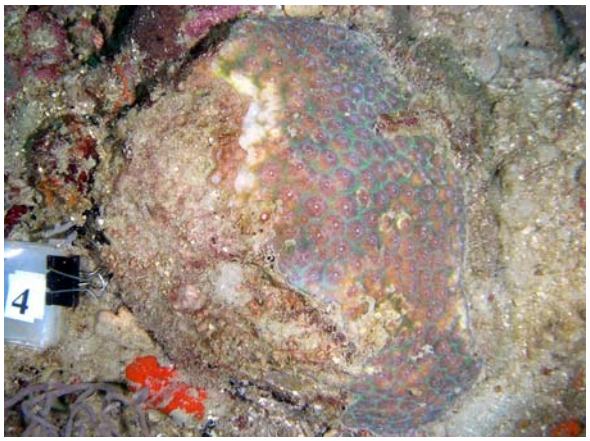

Figure 3b. The same colony at stress level 2 during construction on December 5, 2005, after the passage of three tropical cyclones.

Figure 3c. The same colony showing some tissue loss in the upper left, however, the remaining colony has recovered to stress level 1 during post construction on March 22, 2006

Discussion

Results indicate that in-situ morphological inspection and evaluation of gross changes associated with stress was an effective tool in detecting and adaptively managing impacts to corals during a dredging event adjacent to sensitive coral habitat. Due to the influence of the hurricanes during construction we can easily separate colony stress levels as a result of storms versus dredging. Average stress remained low prior to the first storm. Average stress levels only exceeded level 2 for two of six sites on two occasions at BA1. Threshold shut-down criteria were not exceeded throughout the project.

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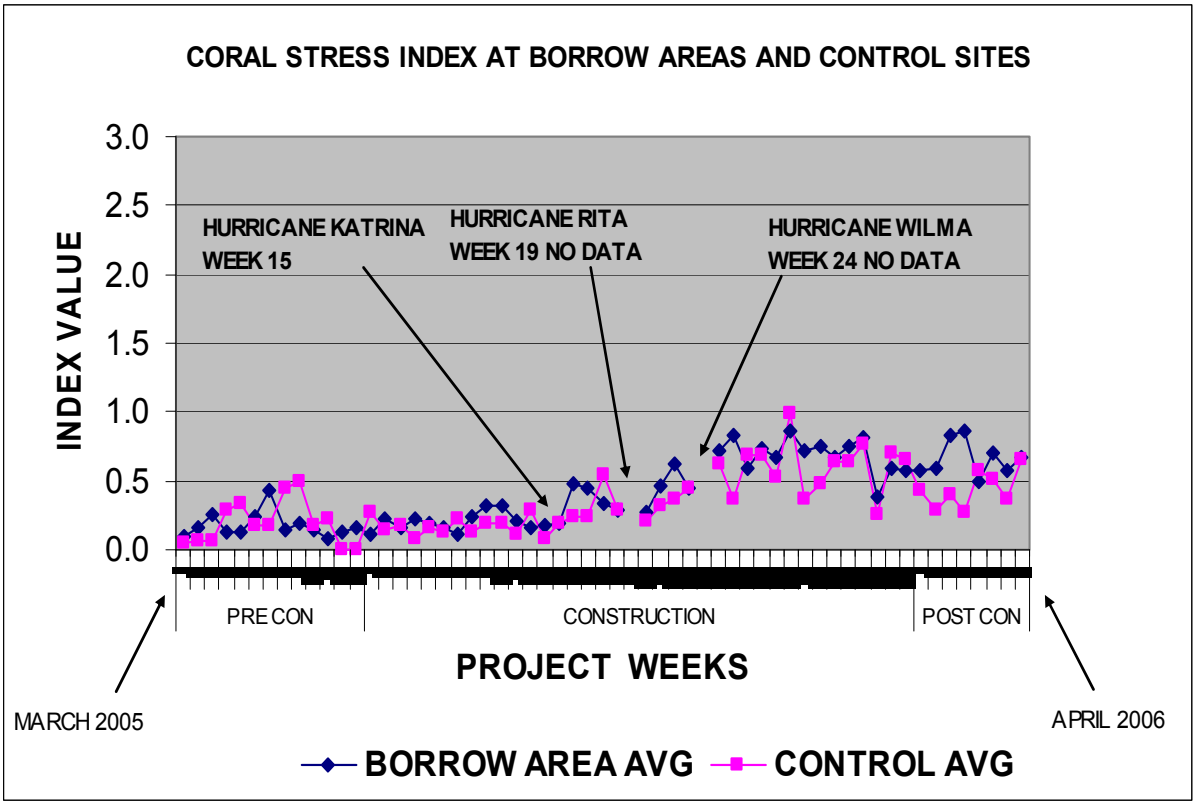

Figure 4. Mean observed stress index values for all corals at all monitoring sites pre- (precon), during (construction), and post-dredging (postcon) . Stress values at treatment and control sites began increasing after the passage of Hurricane Katrina and remained elevated after the passage of Hurricane Wilma throughout the remaining monitoring period.

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The R/V Alpha Helix Expedition: A retrospective analysis of a milestone in coral reef research

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Abstract. The most comprehensive study of a coral reef undertaken until that time, the *Symbios Expedition*, was conducted in 1971 by an interdisciplinary team of ecologists and oceanographers. The study site was at Enewetak Atoll, Marshall Islands, and the *R/V Alpha Helix* along with shore-based facilities provided laboratory and logistical support. Many coral reef researchers are aware that this team-oriented research program made significant contributions to the understanding of coral reef metabolism and function, but the expedition itself has not been documented formally for historical purposes. Here, to assess *Symbios'* published results, we used *Citespace II*, a public domain that provides information visualization methods, bibliometrics, and data mining algorithms in an interactive visualization tool for extraction of patterns in citation data. We found that *Symbios* research results have had a significant scientific impact. Our review of historical reference materials and extensive interviews suggests some of the reasons for the expedition's success: a good science plan, strong and adaptative leadership that emphasized collaborative research, and excellent logistical support lines.

Key words: coral reef, ecosystem metabolism, nutrients, history of science, citation analysis.

Introduction

In the spring of 1971, an interdisciplinary team of marine researchers gathered at Eniwetok Atoll (now "Enewetak"), Marshall Islands to conduct the most comprehensive study of a coral reef undertaken until that time. This expedition resulted in significant advances in the understanding of coral reef metabolism and ecological function.

The name of the expedition, *Symbios*, was chosen as a metaphorical reference both to the symbiotic relationships that characterize coral reefs and to the symbiotic relationships that the late Dr. Robert E. Johannes, the Chief Scientist and driving force behind the expedition, thought should occur among scientists in ecological research. In retrospect, *Symbios* seems to be a clear demonstration of the importance of a well executed collaborative, interdisciplinary approach to undertaking scientific research. Our review shows that this expedition is now regarded as a significant contributor to our understanding of coral reef metabolism and ecological function.

The idea for the *Symbios* Expedition was developed jointly by Robert E. Johannes and Lawrence R. Pomeroy, who were colleagues of Dr. Eugene P. Odum in the University of Georgia's Institute of Ecology. Odum was a renowned pioneer in the field of ecology who, with his equally regarded brother, Howard T. Odum, conducted a remarkable study of coral reef metabolism at Enewetak in the Marshall Islands (Odum and Odum 1955; see also Barile 2004, who has provided a retrospective analysis of the Odums' study). The Odums' monumental monograph notwithstanding, relatively little was known in the 1960s about community metabolism of coral reefs, and Johannes and Pomeroy saw the value of building on the Odums' work. Their aim was to gather an interdisciplinary team of scientists to assess the community metabolism of an entire atoll. The initial objectives of *Symbios*, as presented in the original grant proposal to the U.S. National Science Foundation (NSF), are summarized in Table 1.

Table 1. Initial *Symbios* Proposal Objectives

1. Monitor changes in [chemical and biological] characteristics of seawater as it moves from the open ocean across the windward reef at site one and from the lagoon across the leeward reef at site two.
2. Conduct biological surveys across both reef transects to establish what species may exert quantitatively significant influences on nutrient and/or energy flux in these communities.
3. Determine the effects of the addition of nitrogen and phosphorus on reef community metabolism.
4. Measure changes in oxygen, nitrogen, and phosphorus levels in water flowing across the reef before and after the removal of the large reef herbivores (and coincidentally, the removal of large amounts of nitrogen and phosphorus).

In 1969, with the advice of Dr. W.J. Wiebe and others, Johannes and Pomeroy began the immense task of planning the expedition. According to the

original proposal, the expedition was to take place at Ant Atoll in the Caroline Islands because Enewetak was deemed too small and ill-equipped for such a large operation. As it turned out, it was logistical difficulties with Ant Atoll that led to the switch to Enewetak Atoll, in what was then the U.S. Trust Territory of the Pacific. Logistical support there was better, partially because of the Eniwetok Marine Biological Laboratory (EMBL), supported by the U.S. Atomic Energy Commission (AEC) and directed by Dr. Philip Helfrich.

The expedition was planned as a two-month-long deployment. The *R/V Alpha Helix* (Fig. 1), an NSF-supported research vessel operated by the Scripps Institute of Oceanography (SIO), would remain docked and be used as a floating. An additional group in a shore party would occupy facilities abandoned after the end of the nuclear weapons testing program a little more than a decade before.

Figure 1: The R/V Alpha Helix at Japtan Island pier, Enewetak Atoll. (Photo courtesy S.B. Betzer).

The arrangement worked very well. The ship served as sleeping and eating quarters for half of the scientific party. The ship's laboratories were modern and adequately equipped. Thanks to the creative expertise of the ship's chief electrician, Merle Post, a cable running from the ship provided power to shore buildings, which were used as additional laboratories, and eating and sleeping quarters for the shore party. Thus, the ship's scientific and logistical capabilities were strongly leveraged for a larger scientific party.

The complex logistics of the operation were supported either with direct funding or logistical support from the NSF, the AEC, the SIO, the Janss Foundation, and others. In interviews, *Symbios* participants point to the ingenuity and diligence of Helfrich, Johannes, Pomeroy, and the ship's crew for ensuring the success of the expedition despite the logistical obstacles presented by gathering a 23-person research team (Table 2) in a remote location to carry out such an enormous task.

Table 2. *Symbios Expedition* Scientific Party

Participant	**Role and Home Institution**
Robert E. Johannes[1]	Expedition PI & Chief Scientist; Professor, University of Georgia
Lawrence R. Pomeroy	Expedition Co-PI; Professor, University of Georgia
William J. Wiebe	Professor, University of Georgia
Robert A. Kinzie, III	Postdoc, University of Georgia
James Alberts	Postdoc, University of Georgia
Christopher F. D'Elia	Graduate Student, University of Georgia
William Sottile[1]	Graduate Student, University of Georgia
Michael E.Q. Pilson	Professor, University of Rhode Island
Nelson Marshall	Professor, University of Rhode Island
Susan B. Betzer	Graduate Student, University of Rhode Island
Gregory Telek[1]	Graduate Student, University of Rhode Island
Philip Helfrich	Professor, University of Hawaii
Stephen V. Smith	Postdoc, University of Hawaii
Judy L. Meyer	Graduate Student, University of Hawaii
James E. Maragos	Graduate Student, University of Hawaii
Kenneth L. Webb	Professor, Virginia Institute of Marine Science
William D. DuPaul	Graduate Student, Virginia Institute of Marine Science
Ariel Roth	Professor, Loma Linda University
David Crabtree	Graduate Student, Loma Linda University
James A. Marsh, Jr.	Professor, University of Guam
Robert I. Clutter	Research Scientist, South Pacific Islands Fish. Devel. Commission
Lawrence R. McCloskey	Postdoc, Woods Hole Oceanographic Institution
J. Morgan Wells	Research Scientist, Wrightsville Marine Bio-Medical Laboratory

[1]Deceased

This expedition has, until now, neither been documented for the historical record, nor has its impact on the field been evaluated through citation analysis, which involves the use of citation data for "the construction and application of a wide range of 'bibliometric' indicators of the 'impact,' 'influence' or 'quality' of scholarly work" (Moed 2005). This is not surprising, because scientists, whose primary interest is in the original research they conduct, rarely concern themselves with such things. Thus, we are typically left with only vague notions as to the scientific accomplishments of a given project and the factors that contributed.

The present project aims to paint a picture of the *Symbios Expedition* from its conception and execution through how its scientific results influence our present understanding of coral reefs. We show how its participants now perceive it and most importantly, how *Symbios* science has affected science in general in the last 35 years. As an exercise in the history of science, we hope this

project not only provides better documentation of the *Symbios Expedition*, its successes and the factors that enabled to them, but also leads to other similar studies for other research efforts.

While this project has scholarly aims, we hope that it may also come to the attention of the lay public and students interested in coral reef science.

Approach and Methods

This project had two components, the first being to provide historical documentation using traditional approaches, and the second being to conduct a citation analysis of *Symbios* publications and to assess their scientific impact. (Space limitations here will require us to report on these more fully elsewhere.)

We collected reference materials such as maps, ship logs, and research publications for historical documentation and to gain insight into the logistics of the expedition. Much of this was already in the SIO Library's Archives. The rest will be contributed to that collection. We also gathered from participants over 1600 photos taken during *Symbios* or related to *Symbios*. These provide insight into the living and working conditions of the expedition and help to demonstrate the social dimensions the participants experienced. Finally, we interviewed *Symbios* participants who added detail, perspective, and color to the historical record, and give insight to the reasons for the success of the expedition. Eleven interviews were conducted and transcribed. Participants were asked a series of similar questions but did not have them in advance, nor did they see the interviews conducted for the other participants. For example, we asked each individual what they thought the most significant scientific results of the expedition were, so that their answers could be compared to the results of the citation analysis. Transcripts were made of each interview.

We used citation data and network analysis tools to provide a quantitative understanding of the impact of the science of *Symbios* on scientific understanding. We first assembled a reference list of all papers we could identify that were uniquely or partly the result of research conducted by *Symbios* participants. Both peer-reviewed and non-peer-reviewed literature were included. Using the *ISI Web of Knowledge* (WOK*)*, we compiled citation counts for all peer-reviewed publications that were direct results of the expedition. Unfortunately, book chapters and other non-peer-reviewed publications are not as well covered by the WOK and were not included. The refereed *Symbios* publications were used for analysis of citation frequency and persistency, based on total citation counts and the years in which those citations occurred.

Citespace II, a network visualization tool developed by C. Chen at Drexler University, was used to create citation networks of *Symbios* publications and also to help determine if *Symbios* publications constituted pivotal points in coral reef literature. To create a network, we imported all the resulting publications of a general search into *Citespace II,* and allowed the program to determine which publications were turning points in the literature (pivotal points) based on when, where, how often, and by whom the publication was cited.

Results and Discussion

As was noted above, our approach to assessing the *Symbios Expedition* involved both the more qualitative approach of interviewing participants with the quantitative approach of doing the citation analysis. We felt that this would give us a way to gauge participants' impressions of the scientific accomplishments of the expedition with a wider measure of its impact on the scientific literature.

Oral Interviews. Interviewed participants were in consensus about several key facets of *Symbios*. First, all concluded that the expedition made major contributions to the understanding of the nitrogen cycle and metabolic function of coral reefs. This was verified by citation analysis (see below). Second, those interviewed felt strongly that the interdisciplinary, team approach to research embodied by *Symbios* was a critical characteristic that contributed greatly to Symbios' productivity. Third, the leadership of the late Dr. Robert E. Johannes was frequently cited as a major factor in fostering the scientific vision, cooperative spirit and interdisciplinary collaboration of Symbios. Moreover, interviewees noted that Johannes inspired younger scientists and graduate students, and made a concerted effort to involve women in a major marine science expedition, which was not characteristic at the time. (We believe that the two women who participated in the expedition were likely to have been the only women to have lived on the atoll in the 25-year interval since the Marshallese were expatriated in 1946.) Finally, participants also noted that Pomeroy and Helfrich played essential leadership roles as well.

Although Johannes had died before this retrospective project began, we were fortunate in obtaining an oral interview with him conducted by Dr. Jan Saap. While Johannes did not reflect on the administrative and leadership issues he encountered at all phases of the expedition, it is readily apparent how his enthusiasm for the *Symbios* science was "infectious."

Most interviewees felt that while the scientific objectives of *Symbios* were clearly defined and adhered to throughout the course of the expedition, good communication among participants allowed for "mid-course changes" to improve sampling protocols or take advantage of serendipitous discoveries. The two-month duration of the expedition allowed researchers to modify and repeat field experiments - another major reason cited for the expedition's success. Regular team meetings were held to assess progress and make necessary adjustments. Thus, when flux studies showed reef nitrogen export rates consistent with unexpectedly high rates of nitrogen fixation, it was decided to obtain by rush order the necessary supplies to measure nitrogenase using the acetylene reduction method. Given the logistical support offered by the AEC such as biweekly flights to Enewetak, it was feasible to obtain such critical supplies quickly and for individuals who were not part of the scientific party to join the expedition for a short interval. Thus several scientists from the University of Hawaii also came to conduct shorter complementary studies.

The excellent logistical support provided by SIO, NSF and AEC were mentioned by most interviewed as essential features that enabled *Symbios*' overall productivity. Because Helfrich also directed the AEC's EMBL, he had excellent relationships with key AEC managers in Washington and was able to facilitate logistics.

The other most significant reason for success, according to *Symbios* members, was the fact that the expedition was organized as a team effort, with individuals from widely varying academic backgrounds. At that time, team-oriented ecological studies were uncommon. Pilson said this of the team approach to *Symbios*, "Having a common objective where everybody was there to achieve a goal [was important] and people bring there own interests, their own perspectives to that. But they all wanted to see the enterprise succeed. So there's a certain natural dynamic when people are all keen on making it work." Helfrich said that it was crucial "being able to bring together a lot of scientists, each of whom had a little bit different perspectives and angles on a study like this, and seeing the dynamics of those scientists in the field together." Meyer said the following about of the need for working as an interdisciplinary team in ecological research, "It seems to me that if we are to understand the complexity of the world around us we need to have that diversity of approaches and understanding of the systems. They're complex systems and it's really hubris to think that only one person can truly understand them."

One of the remarkable features of *Symbios* was that it gave younger scientists, including the senior author of this paper, an incredible opportunity to work in a stimulating research environment with top-notch mentors. Meyer, for example, went on to a very distinguished career in stream ecology at the University of Georgia. In her interview, she cited *Symbios* as an important experience in her scientific development and networking that gave her confidence later on. Dr. Susan B. Betzer, on the other hand, said that she learned from *Symbios* that a research career in the ocean sciences was not for her. While she later finished her Ph.D. at the University of Rhode Island (and did include *Symbios*-based research in her thesis), she ultimately obtained an M.D. degree from the University of Miami and still is a practicing physician.

Citation analysis. As we noted above, the participants had their impressions of what the highest impact research conducted was. Citation analysis gave us an opportunity not only to reconcile their impressions with an objective metric, but also allowed us to gain a broader profile of the impact of *Symbios* research.

Symbios participants published nine peer-reviewed papers that related not only to the expedition's major goals but also other topics (Table 3). Many more non-peer-reviewed papers were also published, but they do not lend themselves to citation analysis because they are not well represented in ISI WOK databases.

Table 3. Publications or theses using data from *Symbios* (full citations appear in References).

Betzer (1972)	Pilson and Betzer (1973)
D'Elia (1977)	Pomeroy et al. (1974)
D'Elia (1974)	Smith and Marsh (1973)
Johannes et al. (1972)	Smith (1973)
Johannes and Gerber (1974)	Webb (1975)
Helfrich and Allen (1975)	Wiebe et al. (1975)
Knutson et al. (1972)	

Fig. 2 is a *Citespace II* network created using topic search terms *coral** and *nitrogen*. All publications and corresponding citation data resulting from a Web of Knowledge search using those terms were imported into *Citespace II*, and then analyzed to produce the citation network shown. Nodes represent publications and links represent citations. Rings within nodes represent citations received within time intervals; the larger the node, the more citations it has received from other publications in the network. Pivotal points that are *Symbios* publications or authored by *Symbios* members are noted, with *Symbios* publications noted in bold text. Citation counts

showed high citation rates for all publications, including 738 total citations for these refereed publications.

We do not have sufficient space to report on this topic fully here, but many of the *Symbios* papers are still cited over three decades after publication. Our analysis also showed high persistence for *Symbios'* refereed publications with a mean of 16 citations per publication since the year 2000.

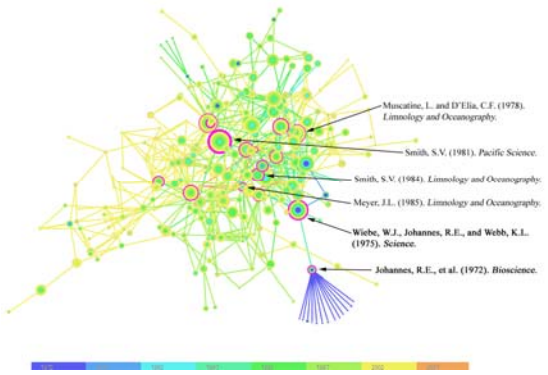

Figure 2. Citespace II network created using topic search terms coral* and nitrogen. (From Harris 2007).

The significant role of serendipity in scientific discovery is also apparent. One unexpected finding was that "Coral Chronometers…," Knutson, Buddemeier, and Smith's 1972 paper on growth bands in corals was the most highly cited publication, largely because of present interests in climate change that were unanticipated in 1971. Smith was only one of two *Symbios* members interviewed that mentioned that paper as a significant result. The research on which it was based was not a core activity of the expedition and was rather done "on the side," as were several other notable *Symbios* publications.

We believe that formal retrospective assessments of research productivity ought to be done more often. Most importantly, such studies provide useful documentation of the value of publicly funded research and the importance that serendipitous discoveries play in basic science. We also encourage more formal study into how large research projects are conducted and managed. Clearly, the team-oriented research of *Symbios* benefited from excellent conceptual and ongoing leadership, good administrative and logistical services, flexible adaptation to changing needs and opportunities, and a remarkably cooperative and team-oriented scientific party. We believe that the *Symbios Expedition* serves as a useful positive example for others to emulate in the future.

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Holocene reef accretion along the north side of Bahia Enriquillo (western Dominican Republic): unique insights into patterns of reef development in response to sea-level rise

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Abstract. Well-exposed reef outcrops along the northern side of the Enriquillo Valley record continuous reef accretion between ca. 9,000 and 5,000 CalBP, when the bay was closed off from the open Caribbean. All the zones of present-day Caribbean reefs are well represented, and facies geometry provides valuable information about both paleo-environmental conditions and changing sea level. Suppressed coral-growth rates, coral distribution/shape, and patterns of bioerosion all point to elevated sedimentation and nutrient influx. Radiocarbon dates, combined with careful stratigraphic analysis, tell a story of early transgression under a regime of rapidly rising sea level followed by regression as sea level slowed and the shallow reef built out over deeper mixed- and massive-coral zones.

Key words: Holocene; fossil reef; reef accretion; sea level, bioerosion

Introduction

The Enriquillo Valley sits on the south-central coast of Hispaniola near the border between the Dominican Republic and Haiti (Fig. 1). During the early Holocene (ca. 9,000 CalBP), the embayment was open to the Caribbean, and coral reefs flourished in a low-energy setting that was exposed to at least periodic intervals of high sedimentation from the steep mountains flanking the bay. By 5,000-4,000 years ago, the bay had closed off due to sedimentation from the Rio Yaque del Sur and possible landslide activity (Mann et al. 1984; Greer and Swart, 2006). Evaporation lowered lake level to its present –42m, exposing the reefs. Arroyos have exposed continuous outcrops that span the entire zonation scheme from shallow, branching corals to deeper-water platy species. This paper describes the general character of the reefs, using a 450-m long exposure at Cañada Honda as the primary example. It then considers the possible environment of deposition based on both reef-community structure and characteristics of individual corals. Finally, it uses the three-dimensional facies pattern, along with radiocarbon ages from the outcrop to address the response of the Enriquillo reefs to rising sea level.

Methods

The outcrop was surveyed using a LaserTech Impulse200 instrument that determined vertical and horizontal intervals to within 1-3 cm. The survey was referenced to permanent markers located by differential GPS (Delorme Earthmate receiver and PostPro softwatre). Reef facies (Fig. 2) were initially defined based on visual estimation of species and point counting of corals at 20-cm vertical intervals along six transects. More detailed measurements were made in 53 meter-square quadrats (4870 data points) along 16 vertical transects. Coral statistics were analyzed within facies to verify or adjust the original boundaries.

Massive corals were divided into three primary shapes: hemispherical (the most common shape on modern reefs), conical and columnar. The shape of the colonies encountered in the quadrats were recorded and used to tie shape preference by species and overall within particular outcrops. A well-defined storm layer within the outcrop (Fig. 3) provides a time line that was used to characterize the morphology of the reef. It also provided an instantaneous surface below which we examined coral taphonomy and bioerosion by coral species and shape. Radiocarbon dates from corals just beneath the storm layer across the outcrop confirm the synchronicity of the event and date it at 9,500-9,000 CalBP.

Results

A total of 27 coral species (plus two *Millepora* species) were identified in the Enriquillo Valley reefs. The relative abundance of the most important species

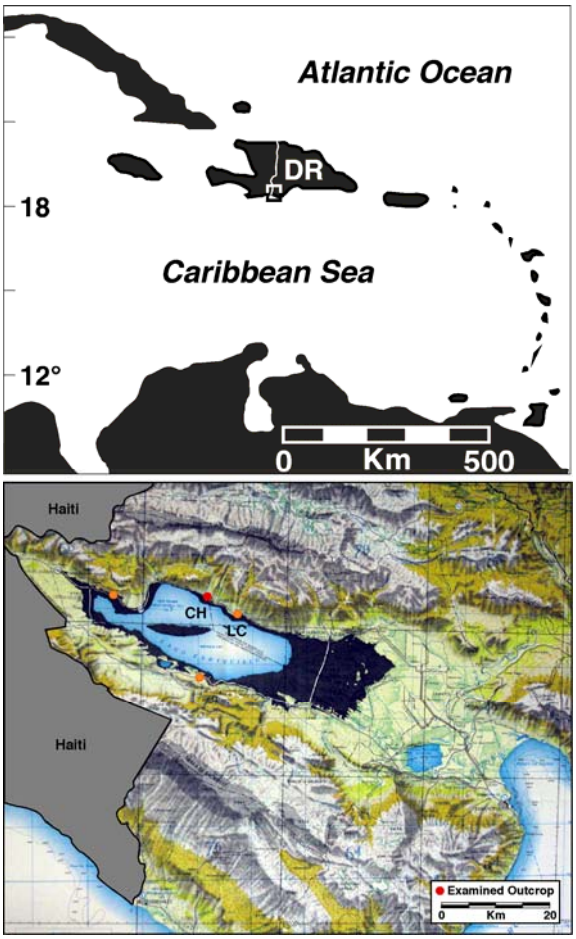

Figure 1: Map showing the location of the location of the Enriquillo Valley (box in upper) and the outcrops at Cañada Honda (CH) and Las Clavellinas (LC) in the Enriquillo Valley (lower).

Table 1. Summary of major species and overall coral diversity by facies.

Facies	Massive	Mixed	Platy	M3
Agaricia spp.	0.7	2.7	2.4	1.7
C. natans	3.7	8.7	0.9	1.5
Dichocoenia sp.	1.1	0.9	0	0.4
Madracis spp. (2)	1.2	0.2	1.0	1.6
*M. faveolata**	10.9	4.6	7.4	3.8
M franksii/cavernosa	0.1	0.0	0.0	23.7
Eusmilia spp. (2)	0.3	0.4	0.7	0.4
S. radians	0.8	4.6	0.6	2.9
S. siderea	43.2	35.5	14.7	21.1
Stephanocoenia sp.	3.0	0.1	4.4	0.5
Total Other Coral	2.3	1.7	2.0	0.0
Total Molluscs	0.4	0.3	0.0	0.2
Total Sed/Rubble	32.3	40.5	65.7	42.1
No. of Species	14.0	18.0	12.0	12.0
Avg. Species/Transect	8.1	7.3	5.5	3.8

* includes a variant with smaller polyps: aka Mini-*Montastraea*

is summarized in Table 1. The facies exposed in the outcrop represent all the major coral zones found on modern Caribbean reefs (Fig. 2). Within the branching coral zone, *Acropora palmata* is replaced by *A. cervicornis*, probably because of the low wave

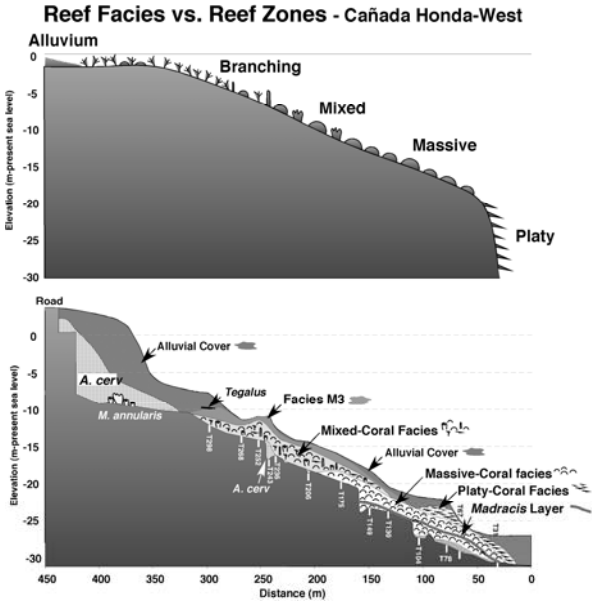

Figure 2: Cross-sections of a modern Caribbean reef (upper) and facies within the outcrop at Cañada Honda (lower)

Figure 3. Photograph of a storm layer dominated by *Madracis* spp. Note how many of the corals lean toward the right (i.e., downslope), reflecting disruption as the detrital layer was put in place.

energy in the protected embayment. Most of the coral appears to be either in place or simply compressed by collapse and burial. The oldest *A. cervicornis* colonies are found midway along the outcrop and the species extends to the uppermost part of the exposure, reflecting gradual transgression as sea level rose. Coral diversity was greatest in the mixed-coral zone, which was dominated by *Siderastrea siderea* and lesser amounts of *Colpophyllia natans* and *Montastraea* spp. (Table 1; Fig. 4). Corals in the deeper massive-coral zone were more evenly split between *Montastraea* spp. and *Siderastrea* spp. In the platy coral zone, the dominant species are *S. siderea* and *M. franksii*. Sediment made up 32-66% of the reef interior.

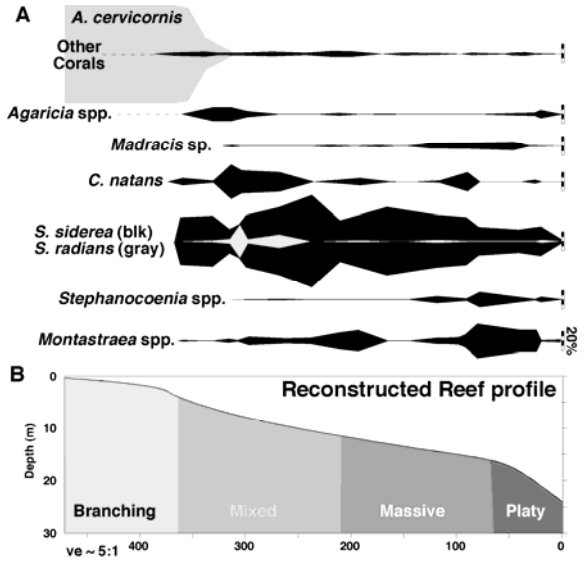

Figure 4: Reconstructed reef profile for the reef exposed in Cañada Honda (below). The coral abundance within facies and in the corresponding environments on open Caribbean reefs is shown by the Mae West diagrams at the top.

The massive-coral zone is interrupted by a layer of *Madracis* sp. (Fig. 3) that was initially deposited during a storm event 9,400-9,000 years ago. While there is evidence of recolonization and possibly additional debris emplacement later on, the base of the layer undoubtedly reflects a short-lived interval that represents a time line through the reef. This layer allows us to reconstruct the morphology and paleo-depths along the reef at that time. It also provides an opportunity to assess the relative importance of live versus dead coral along the reef at any one time. Preliminary studies based on the taphonomic condition of the buried corals (pristine or near-pristine corals are assumed to have been alive at the time of burial; those with significantly altered or encrusted surfaces were scored as "dead") infer that approximately half of the coral community was alive before the storm event that buried the entire forereef profile. We can use this value to "adjust" the quadrat data shown in Table 1 to approximate live-coral cover on an equivalent modern reef. Based on these numbers, live coral cover probably ranged from nearly 50% in the branching-coral zone to between 16 and 33% along the rest of the reef. Whether we use the raw quadrat data or the numbers adjusted by the taphonomic estimates, the percent cover along the Enriquillo Valley reefs approaches what is seen on modern Caribbean reefs, despite the protected nature of the embayment and the high likelihood of elevated sedimentation and nutrient loading.

Higher sedimentation is supported by three lines of evidence. First, the growth rates of *Siderastrea* spp. and *Montastraea* spp. are generally depressed

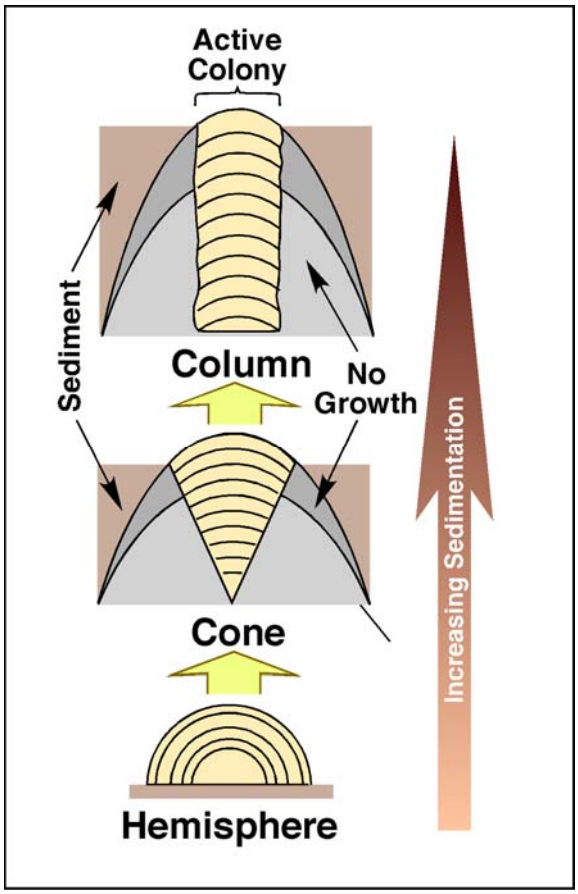

Figure 5. Effects of increasing sedimentation on coral shape in Bahia Enriquillo. On normal, oligotrophic reefs exposed to less sediment, hemispheres dominate. As the rate of sedimentation increases, polyps near the base of the colony will increasingly be overcome and lateral colony extension will be reduced. As a result, coral shape will gradually shift toward conical and columnar forms until a point at which sedimentation becomes so severe that the colony ceases to be viable and is totally overwhelmed.

(1-3 mm/yr) compared to the published rates for the same species at similar depths in the open Caribbean (5-10 mm/yr). Second, coral shapes are rarely hemispherical in outcrop. Conical and columnar growth forms are much more common. It is presumed that as sedimentation increases, lateral colony extension will be more difficult (Fig. 5). As a result, coral shape will shift from hemispherical to conical to columnar as coral bases are increasingly buried by sediment. In Cañada Honda, *Montastraea* spp. tends to be columnar, compared to a more conical growth form for the more sediment-tolerant *Siderastrea* spp. (Fig. 6). In an outcrop near Las Clavellinas (LC in Fig. 1), sedimentation rates are higher. In response, *Siderastrea* spp. shifts to a columnar form and *Montastraea* spp. is virtually absent, following the expected relationship between form and sedimentation. Finally, bioerosion in the Cañada Honda corals is higher and involves different

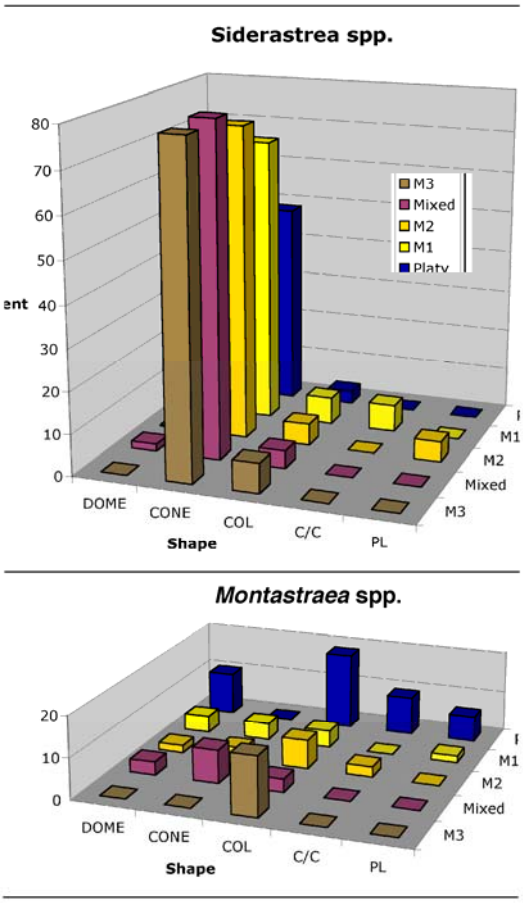

Figure 6. Graphs of coral shapes associated with the two dominant genera in the reef. The more sediment-tolerant species of *Siderastrea* tend to be conical, reflecting some difficulty with sedimentation. *Montastraea* spp. are even less tolerant and show a greater tendency toward columnar forms.

organisms than are typical of open-Caribbean settings. Bioerosion showed a general decrease with paleo-depth, similar to what has been found on open-Caribbean reefs. However, on a more general level, the difference between the borer communities at the two sites were statistically different at a value of $\alpha<.001$. The dominant borers in the Bahia Enriquillo corals were lithophagid molluscs. This contrasts dramatically with the greater importance of sponges in corals sampled from the US Virgin Islands (Fig. 7). This is consistent with past studies that have tied boring bivalves to well-documented high-nutrient environments (Highsmith 1980; Sammarco and Risk 1990; Perry and Hepburn 2007). Another indication of both high and episodic sediment stress is the abundance of colonies the reflect periods of burial separated by intervals in which the coral could build laterally over the remaining surface (Fig. 8).

On a larger scale, the overall facies geometry that is preserved in the outcrop (Fig. 2) reveals a change in accretionary style over time. Along the lower and

Figure 7. Photographs of coral slabs from Cañada Honda (left) and Cane Bay (St. Croix, USVI – right). In the more protected and higher-sediment/nutrient environment of Bahia Enriquillo, lithophagid molluscs dominated, compared to a borer community dominated by sponges in the open Caribbean.

central portions of the outcrop, coral date between 9,000 and 7,000 CalBP, a time when sea level was rising very rapidly (Fig. 9). Facies generally deepen upward (from massive to platy). This pattern is repeated in the central part of the outcrop, where facies change upward from massive to mixed corals– see Fig. 9 lower right photo). Later on, as sea level slowed, a gradual shift toward a regressive pattern emerges.

Discussion

The spectacularly exposed reefs in the Enriquillo Valley provide a unique opportunity to examine the internal structure of Holocene reefs at a level of detail impossible in cores. The species diversity within these reefs was surprisingly high, given the low energy and high sedimentation they experienced. This presumption is supported by the shift from *A. palmata* (which has no biological mechanism for sediment removal and relies on wave energy) to *A. cervicornis*, as well as retarded growth rates (1-3 mm vs 5-10 mm), the general lack of hemispherical colonies (except in *Colpophyllia natans*), and the dominance of lithophagid molluscs that require high nutrient levels to support their metabolic needs.

This high diversity flies in the face of modern paradigms that stress low coral abundance and diversity on reefs exposed to high sedimentation. The "pancake" morphology (Fig. 8) that is common

Figure 8. Photograph of "pancake" coral morphology. Each interruption in upward extension is interpreted as partial burial by sediment during storms that either delivered more sediment from land or, less likely, shifted it in from adjacent environments.

in all outcrops reflects high but periodic sedimentation. The present climate is very dry, but is seasonally interrupted by intense, flashy rains typical of a desert region. If this climate prevailed in earlier Holocene times, the high coral diversity may reflect clearer water in the intervals between rains. Greer and Swart (2006) have proposed variable rainfall on a decadal scale. If this extends to shorter periods of time, then the higher-than-expected diversity and abundance numbers may reflect "longer intervals of boredom separated by brief episodes of terror". Superimposed on this were the much larger events reflected in the storm-debris layer that is dominated by *Madracis* sp. (Fig. 3). Alternately, we may have to re-examine our existing ideas about sedimentation and coral diversity. Perhaps reefs that developed in a regime of long-term sediment stress were able to adapt, in contrast to the situation on modern reefs exposed to anthropogenically induced conditions.

Whatever the answer to this dilemma, the exposures at Cañada Honda allow us to address these and similar questions at a level of detail that was previously impossible. On a larger scale, the pattern of early transgression followed by a shift to regression as the rate of sea-level slowed provides strong support for the new and simplified Holocene reef model proposed by Hubbard (this volume).

Acknowledgement

This study was supported with funds from the Petroleum Research Find of the American Chemical Society, the Keck Geology Consortium and Oberlin College. Much of the work described above comes from careful work done by Oberlin undergraduates, in particular, Allicia Davis, Greg Lawson, Ali Stein, Josh Brewer, Jonathan Meed, and Jesse Oram. Karla Parsons-Hubbard helped with the taphonomic analyses, which have subsequently been improved by Hal Lesinsky

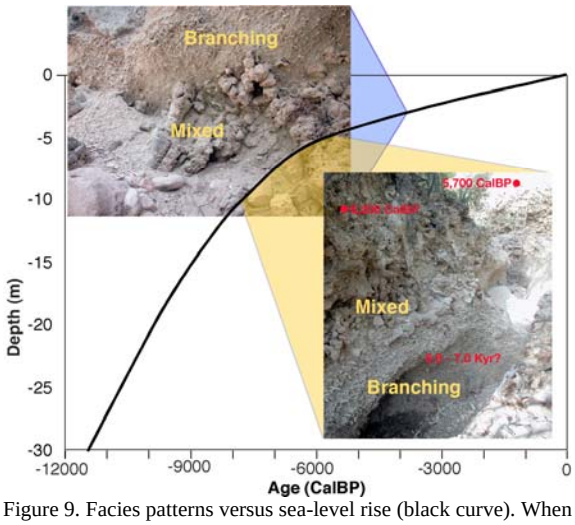

Figure 9. Facies patterns versus sea-level rise (black curve). When sea level was rising more rapidly, paleo-environments deepened upward (lower right photograph). As sea-level rise slowed, the shallow branching facies migrated seaward over the deeper mixed-coral facies. This demonstrates a pattern of transgression before 6-7,000 years ago, gradually shifting to regression.

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PROCEEDINGS OF THE

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Shallow seismic profiling survey on postglacial fore-reef near the present-day northern limit of coral reef formation in the northwestern Pacific

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Abstract. Shallow seismic profiling survey, bathymetric mapping, and ROV (remotely operated vehicle) observations were carried out to the east of northern Amami-o-shima Island (28˚24'N, 129˚38'E). The survey area is located in a northern part of the Ryukyu Island Arc and close to the present-day northern limit of coral reef formation in the northwestern Pacific. Irregularly-shaped mounds occur in the northeastern part of the survey area. These mounds appear to be composed internally of postglacial reef deposits which are covered with modern coral assemblages. Seismic profiles show that channels several hundred meters long are developed on the western margin of the mounds. The channels may correspond to eroded valleys formed during the last glacial period and are overlain by stratified sediments. This indicates that coarse siliciclastic sediments derived from Amami-o-shima Island were trapped in the channels and that limited sediments were transported into the eastern part, which may have allowed coral reef formation in that part during the last deglaciation.

Key words: Seismic profiling survey, Ryukyu Island arc, Postglacial, Fore-reef, Northwestern Pacific.

Introduction

The Ryukyu Island Arc extends from Kyushu to Taiwan, a distance of 1200 km, along the Ryukyu Trench where the Philippine Sea Plate is subducting beneath the Eurasian Plate (Fig. 1). The Okinawa Trough, a back arc basin (Shinjo et al. 1999), formed behind the Ryukyu Island Arc in the late Pliocene to early Pleistocene (Sibuet et al. 1998 and Park et al, 1998). The formation of the Okinawa Trough is one of the most important factors for initiation and development of coral reefs in Ryukyus because it has trapped siliciclastic sediments from the Eurasian Continent, which changed paleoceanographic conditions in this area from the 'mud sea' to the 'coral sea'. The warm Kuroshio Current enters the Okinawa Trough and flows northeastwards along the Ryukyu Islands. The current brings larvae and planula of hermatypic corals as well as heat. This enables coral reef formation throughout the Ryukyus, although the islands lie at relatively high latitudes compared with other reef sites (Iryu et al. 2006).

In this study, we present high-resolution seismic profiles of modern fore-reef on the shelf of the Ryukyu Island Arc. The survey area was located in a northern part of the Ryukyu Island Arc, northeast of Amami-o-shima Island. The survey area lies close to the present-day northern limit of coral reef formation in the northwestern Pacific which is situated at Tane-ga-shima Island (Iryu et al. 2006). This survey aims to delineate the lateral extent and depth distribution of coral reefs formed during the last glacial and postglacial periods at a marginal region of the reef province in the northwestern Pacific and to unravel the factors controlling initiation of coral reefs during the last deglaciation.

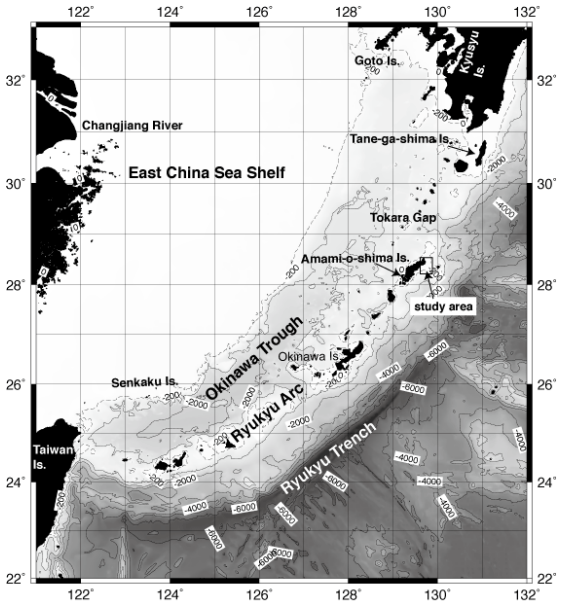

Figure 1: Bathymetric map of the Ryukyu Island Arc. Note the study area off Amami-o-shima Island which is close the present-day coral reef formation located at Tane-ga-shima Island.

Methods

The survey cruise was carried out by Dai-hachi Shofuku-maru (fisherman's boat with gross tonnage of 6.54). Seismic profiles were acquired by AA300 Boomer Plate system with 8 channels digital streamer cable. Data were collected using a 2-second-firing rate. Data were logged to SEG-D format using a CNT-2 digital acquisition system. The survey lines in a WNW-ESE direction ranged in length from 2 to 2.5 km, where water depth varied between 10 and 60 m (Fig. 2). The interval of the lines was about 100 m. The data were stacked before interpretation at onshore survey.

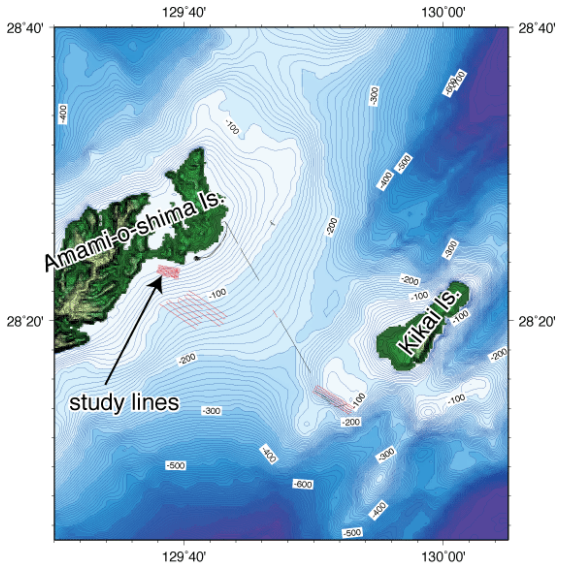

Figure 2: Seismic and bathymetric survey lines in the study area. The data of this study were obtained from shallow areas ranging in water depth from 10 to 60 m, to the east of Amami-o-shima Island.

Bathymetric surveys were conducted using a SeaBat 8101 multibeam echosounder along the seismic survey lines. After the seismic and bathymetric surveys, we obtained the video images using remotely operated vehicle (ROV) LBV150 SE (Nippon Kaiyo Co., Ltd). The ROV was deployed in the several areas on and around postglacial to modern fore-reef, where water depths ranged from 7 to 45 m and provided high-quality visual images of the reef deposits.

Results

The ROV video images and bathymetric maps indicate that the northeastern part of the survey area is characterized by presence of irregularly-shaped mounds (Fig. 3A). These mounds appear to be composed internally postglacial reef deposits which are covered with modern coral assemblages. In contrast, a flat seafloor extends in the western and southern deeper areas, consisting mainly of coarse sediments (Fig. 3B).

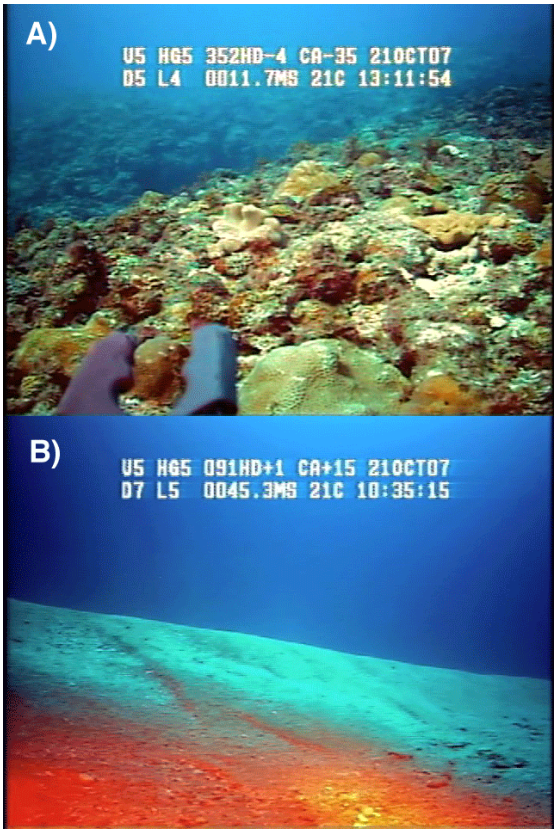

Figure 3: ROV video images. A) Modern coral reef at a water depth of 11.7 m in the eastern part of survey area. B) Flat sea floor composed chiefly of coarse sediments at a water depth of 45 m.

The reefs in Fig. 4 extend along the coastal line with a total span of > 1.5 km in the northeastern part of survey area. Spurs and grooves are arranged perpendicularly to the seaward margin of the reefs (Fig. 4) and extend for 1 km off the margin. The

grooves are several tens meter width and several meter deep. The base of the spurs and grooves are < 50 m water depth. We found similar irregularly-shaped mounds covered with modern corals in southern part of study area, where water depth is < 30 m. The mounds rimmed by spurs and grooves are < 150 m in a total span.

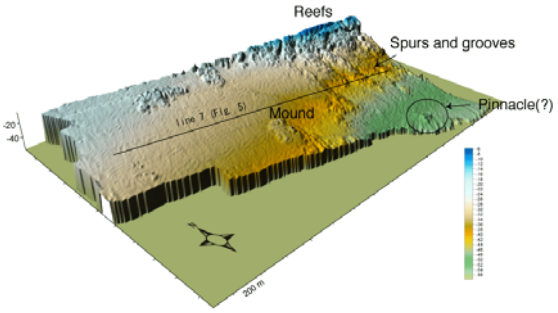

Figure 4: Oblique view of survey area. Irregularly-shaped mounds occur in northeastern area. The spur and groove structures with a N-S direction were observed. Note the locations of seismic profile in Fig. 5.

High-resolution seismic profiles show a distinct, irregularly-undulated reflector which can be traced throughout the survey area (Fig. 5). The reflector, beneath which the profiles are not clear, is overlain by the stratified sediments. The maximum thickness of the stratified sediments is 60 ms in two way travel time. The sediments showing continuous parallel reflections unconformably onlaps the irregularly-undulated reflector.

Channels are found on the western margin of the eastern part of the survey area where the irregularly-shaped mounds occur. The channel shown in Fig. 5 is 100-200 m in wide and can be as deep as 45 m if we assume that constant velocity of sediments is 1500 m/s. The channels have a north-south direction extending over several hundred meters. Channels of a similar size to this are found on other lines in the survey area (Fig. 6).

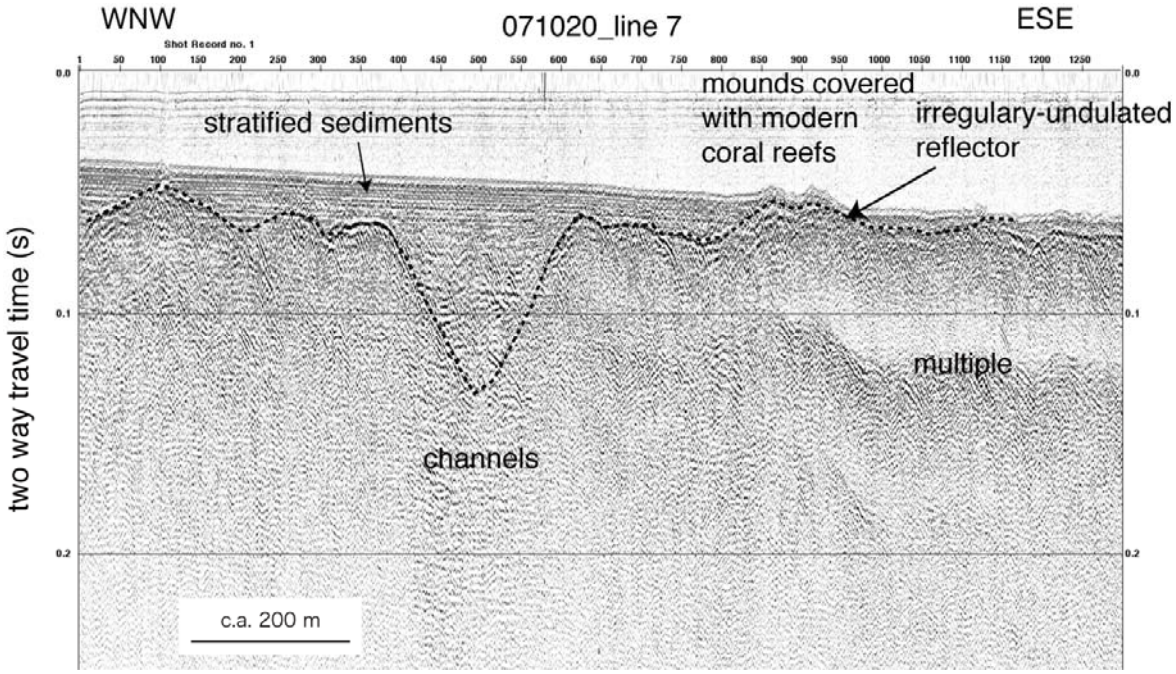

Figure 5: Stacked profile of 8ch MCS off eastern Amami-o-shima Island (Figs. 2 and 4). A distinct, irregularly-undulated reflector is recognized throughout the survey area.

Discussion

Unclear internal structures below the distinct, irregularly-undulated reflector contrast well with the overlying parallel continuous reflections. This indicates that the stratified sediments lie unconformably on the erosional unconformity surface (represented by the irregularly-undulated reflector) formed by subaerial exposure during the last glacial period. The ~45 m deep channels are likely to correspond to eroded valleys formed during this period, as well. The base of the stratified sediments is characterized by an onlap configuration, which suggests that the sediments with parallel stratification accumulated on the last glacial erosional surface during the postglacial sea-level rise. The thickness of the stratified sediments decreases eastward (Fig. 5); this indicates the sediment supply to the survey area came from the west (Amami-o-shima Island) during the postglacial period. Several channels are found on the western margin of the eastern part of the study area in which irregularly-shaped mounds composed of postglacial reefs covered with modern coral communities occur (Fig. 6).

Our data suggests that the coarse silisclastic

sediments derived from Amami-o-shima Island were trapped in the channels and this limited the transportation of sediments into the eastern part. This may have allowed coral reef formation in the eastern study area during the last deglaciation.

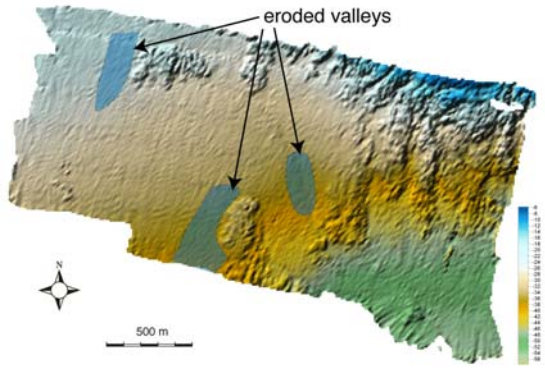

Figure 6: Bathymetric map showing the distribution of eroded valleys (blue area).

Conclusions

1. Internal structures of the modern fore-reef were revealed using a multi channel high-resolution seismic survey.
2. The ROV video images and bathymetric maps clearly indicate that the northeastern part of the survey area is characterized by presence of irregularly-shaped mounds of modern coral reefs and that the flat seafloor, consisting mainly of coarse sediments, extends in the southern and western area.
3. A distinct, irregularly-undulated reflector which may represent erosional surface formed in the last glacial period is recognized throughout the survey area.
4. The channels which may correspond to eroded valleys formed during the last glacial period are developed on the western margin of the eastern part of the survey area where the irregularly-shaped mounds occur.
5. The coarse sediments derived from Amami-o-shima Island were trapped in the channels, which limited sediment transportation into the eastern part of the study area. This may have allowed coral reef formation in this area during the last deglaciation.

Acknowledgement

We express our thanks to Captain Matuyama of No. 8 Shofuku-maru and the Amami-o-shima Fisheries Cooperative Association for assistance for our safety survey. We also thank Dr. F. Murakami of GSJ-AIST and Mr. T. Ando of Nippon Kaiyo Co. Ltd for technical support of Boomer systems and that of ROV operations, respectively. Our survey was financially supported in part by the grant from AESTO and JAMSTEC for the preparation of IODP proposal.

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The Darwin Point: a conceptual and historical review

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Abstract. The term "Darwin Point" is defined as the geographic or depth limit (threshold) beyond or below which coral reefs drown. Reef drowning occurs when net production of $CaCO_3$ or vertical accretion of the reef no longer keeps up with relative sea level. If sea level is rising very fast, then reef drowning can occur even if there is very low vertical accretion. If present ecological conditions were to change, due to a rise or fall in sea-level, geophysical uplift or subsidence, or Global Climate Change, the geographic location or depth limit of a Darwin Point would also change. In this paper, the history of the Darwin Point concept is reviewed and several examples are given of reefs and atolls that have drowned having exceeded a Darwin Point threshold. Such appears to be the case for: 1) guyots beyond the northwestern end of the Hawaiian Archipelago, 2) atolls that crossed equatorial latitudes due to plate movement in the Pacific during Cretaceous Time, and 3) many drowned reefs extant at the present time; a result of sea-level rise since the last Glacial Maximum 21,000 years ago.

Key words: Darwin Point, coral reef drowning, coral growth, bio-erosion

Introduction

Darwin Points define thresholds of reef drowning. A Darwin Point is reached when the net production or the accretion rate of $CaCO_3$ by reef building corals no longer keeps pace with relative sea level. In 1972, K. Chave (Chave et al. 1972) described net production of $CaCO_3$ as the $CaCO_3$ permanently retained by the reef. Reef drowning occurs when production rates due to coral growth are exceeded by rates of bio-erosion and physical induced loss, and/or rates of subsidence or sea level rise.

Application to the Hawaiian Archipelago

The Hawaiian Islands consist of a long and linear chain of volcanic islands, coral islands and atolls, which are slowly subsiding and gradually drifting (by tectonic displacement) to the northwest from tropical into subtropical latitudes and cooler water. The growth rate of reef building corals surrounding the islands should therefore decrease with increasing latitude within the island chain. At one point the growth of corals should no longer support and sustain atolls at sea-level. This point defines a threshold for atoll development. The research needed to test this hypothesis was conducted between 1978-1981 (Grigg 1982). Rates of gross production of $CaCO_3$ per unit area were measured from the first island of Hawaii to the last island in the Hawaiian Archipelago, Kure Atoll, a range of latitude of about 10 degrees (~19.5–29.5 N. Latitude). The data were collected at comparable stations and depths. All stations were located off southwest exposures off the islands or banks at depths of 10 m in areas of maximum reef development.

At each station, coral growth was measured by averaging the width of at least ten annual growth bands (Knutson et al. 1972) in skeletal cross sections of ten colonies of the most abundant species, *Porites lobata*. Colony mass accretion in kg $CaCO_3$ was calculated as the product of mean linear growth rate and mean colony density. Mean values of mass accretion for colonies of *P. lobata* were then computed for each island and multiplied by measures of mean coral cover for all species of coral, producing mean rates of accretion due to all corals in Kg $CaCO_3/m^2$/year (Fig. 1). *P. lobata* is considered representative of other Hawaiian coral because of its massive growth form, and because it is intermediate in growth rate (Buddemeier et al. 1974). It is also the dominant framework builder in the Hawaiian Archipelago (Grigg 1983).

All measures of growth were taken along axes of maximum growth in each colony. Estimates of coral bottom cover were taken from optimal areas, where rates of accretion were considered estimates of maximum gross production for corals. In optimal environments in Hawaii, rates of erosion and dissolution are small and gross production is a reasonable approximation of net production. In fact, estimates of calcification based on the method used here agree well with measures of net calcification based on alkalinity depression for comparable areas in Hawaii (Kinsey 1979).

The results of the study showed that islands drown at a point very close to 29 degrees north latitude, although consideration of historical patterns of sea-level change or climatic change may have periodically shifted this threshold, dubbed the Darwin Point, northwest or southeast in response to changing sea level or thermal structure (Grigg 1997).

The data illustrates that coral reef accretion declines linearly as a function of latitude from Hawaii in the southeast to Kure Atoll in the northwest (Fig. 1). Mean accretion rates of the reefs due to coral growth alone at the southeast and northwest extremes of the Archipelago were 11 mm/yr (15 kg $CaCO_3/m^2/yr$) and 0.2 mm (0.3 kg $CaCO_3/m^2/yr$), respectively. The accretion rate at Kure Atoll of 0.2 mm/yr is very close to zero and taking into account biological and physical erosion, it is reasonable to conclude that it is virtually zero. On a longer time scale, Kure Atoll will likely undergo drowning and join 12 other drowned atolls (guyots) that exist in a linear sequence to the northwest. These drowned features are known as the Emperor Seamounts and all were once thriving atolls southeast of a Darwin Point latitude. They range in age between 30 and70 Ma. Their geomorphology and fossil summits strongly suggest they were all thriving atolls before drifting northwestward past a paleo-Darwin Point. The Darwin Point thus separates the Hawaiian Archipelago into two approximately equal halves; islands that are presently at or above sea-level and those that have drowned. All sub-aerial islands are southeast of the Darwin Point; all drowned guyots are northwest of the Darwin Point.

Application to atoll drowning near the equator during Cretaceous Time

In 1993, the Ocean Drilling Program (ODP), legs 143 and 144, recovered cores from seven guyots in the northwest Pacific Ocean. Analysis of the cores showed that these volcanic edifices underwent prolonged volcanism (128-84 Ma), followed by subsidence, accumulation of shallow-water coral reef carbonates, emersion due to a sea-level fall, and then continued subsidence, submergence and eventual drowning. According to Peter Flood (1999), five of the seven guyots drowned at latitudes within 8 degrees of the equator having been transported there by plate movement during the Mid-Cretaceous. Flood ascribed the drowning events to be the result of the atolls entering a crisis zone (Darwin Point) near the equator where upwelling produced nutrient rich waters that inhibited carbonate production (Figs. 2 and 3). Flood also suggested that sea-level rise could have been an additional factor causing the drowning of these atolls. In support of this hypothesis, Schlanger et al. (1981) concluded that wide-spread mid-plate volcanism between 100-70Ma caused thermally induced uplift of the Pacific and Farallon plates leading to global Cretaceous transgressions (Fig. 4). Thus a shifting Darwin Point near the equator in the Western Pacific Ocean could be the explanation for the drowning of these and other atolls that were transported through equatorial waters by plate motion during Mid-Cretaceous time.

Application to a depth limit for the accretion of coral reefs

It is well known that the critical depth for the formation and accretion of coral reefs in optimal environments is generally found at depths between 30-50 m (Grigg 2006, Grigg and Epp 1989, Hopley 1982, Darwin 1962 Reprinted). In environments less than optimal, critical depth would be expected to be shallower. Critical depth as described herein may conceptually be viewed synonymously with a Darwin Point in the third dimension, e.g., a vertical Darwin Point. While this synonymy is somewhat semantic, the factor or factors producing a Darwin Point are not.

Because calcification rates in hermatypic corals are significantly greater than species lacking zooxanthellae, light has long been considered the primary factor setting the lower depth limit for the reef building process (Barnes and Chalker 1990, Wells 1957). This is particularly true for gross production of $CaCO_3$ by reef building corals (Chave et al. 1972). However, if depth limitation depends on the net carbonate production retained by the reef, then many other intervening factors such as temperature, turbidity, bio-erosion and mechanical losses due to waves must be considered. In whatever case, when net production is zero (assuming constant sea-level change) the over-all reef is at a drowning threshold or vertical Darwin Point.

Recent measures of a vertical Darwin Point have been made off Lahaina, Maui in the Au'au Channel in the Southeast Hawaiian Islands (Grigg 2006). The threshold depth of reef drowning there was found to exist at 50 m (Fig. 5). Below 50 m, the rate of bio-erosion of the holdfasts of the major reef building species, *P. lobata*, exceeded the growth of basal attachments causing colonies to detach from the bottom. Continued bio-erosion further eroded colonies until they were dislodged by bottom currents leading to their breakdown and ultimate formation of carbonate rubble and sand. Coral colonies of species that grow deeper than 50 m in the Au'au Channel, (some down to 120 m [Kahng and Maragos 2007]), do not permanently attach and accrete. A Darwin Point is reached at 50 m in the Au'au Channel, off Maui, because gross carbonate production minus bio-erosion is zero (Fig. 5). In an analogous manner, biological and mechanical erosion at Kure Atoll in the NWHI reduces a positive net rate of $CaCO_3$ production there to zero, giving way to a zoogeographic Darwin Point near 29° North latitude. Similarly, Darwin Point thresholds were surpassed many times along the outer edge of the Great Barrier Reef during the last transgression (Hopley 2006).

In summary, Darwin Points are found at both latitudinal and depth limits where the net production of $CaCO_3$ retained by the reef is zero relative to sea level. The critical depth for the entire reef is a Darwin Point, but it may be deeper for individual corals. Historically,

Darwin Points have existed since the evolution of reef building corals at the beginning of the Mesozoic Era. They have set both the zoogeographic and depth boundaries for coral reefs for 240 Ma and continue to do so. Interestingly, in 1837, while viewing the island of Moorea from an elevation of about 300 m above Point Venus on the island of Tahiti, both limits were conceived conceptually by Charles Darwin himself (Darwin 1851).

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Effects of ocean acidification and increased temperature on skeletal growth of two scleractinian corals, *Pocillopora meandrina* and *Porites rus*

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Abstract
For tropical corals, the projected decreases in seawater pH due to increasing atmospheric pCO_2 are predicted to reduce calcification by 8% to 40%, but little is known of the interactions with other physical conditions, or the effects on other biological processes. This study tested for the interactive effects of pH and temperature on the photosynthesis and calcification of corals using experiments in which pH was reduced (7.8), simulating year 2100 pCO_2 levels, and was crossed with two temperatures (27 ºC and 29 ºC). In 14-day incubations with *Pocillopora meandrina* and *Porites rus*, calcification was depressed ≥ 50% at 27 ºC and 700 ppm CO_2, compared to 27 ºC and 395 ppm CO_2, but was not depressed by high pCO_2 at 29 ºC; dark-adapted maximum quantum yield of PSII (F_v/F_m) was unaffected by the same treatments of temperature and pCO_2. Thus, while F_v/F_m in *P. meandrina* and *P. rus* is resilient to slight increases in temperature and high pCO_2, calcification shows temperature-dependent sensitivity to high pCO_2, notably with high temperature apparently conferring resistance against the short-term effects of high pCO_2.

Key words: Scleractinia, Calcification, Climate change, Photosynthesis, Temperature,

Introduction

The foundations of tropical reefs rely on the prodigious capacity of symbiotic scleractinians to calcify and to fix large amounts of carbon (Hoegh-Guldberg 2005). Rapid calcification by corals plays a critical role in coral reef function, because it leads to the formation of a massive, wave resistant framework that provides habitat for many taxa (Idjadi and Edmunds 2006). Understanding the factors controlling coral calcification is critical to project coral community structure into the a future strongly impacted by global climate change (GCC), and to elucidate the implications of global climate change (GCC) for the taxa that rely on coral structure as habitat.

Already, many coral reefs are in a state of decline (Hoegh-Guldberg et al. 2007) due in large part to the effects of multiple anthropogenic disturbances acting locally (Jackson et al. 2001) as well as regionally (Hoegh-Guldberg et al. 2007). Predictions of the biotic effects of GCC have identified temperature and partial pressure of atmospheric CO_2 (pCO_2) as factors with serious implications for marine ecosystems, specifically through increasing acidity of seawater (i.e., ocean acidification, or OA), and rising ocean temperatures (IPCC 2007). Current pCO_2 levels of ≈350-380 ppm are predicted to exceed 700 ppm by the year 2100, if "business-as-usual" CO_2 emissions are maintained (IPCC 2007). Such a dramatic increase will cause substantial declines in the pH, aragonite saturation state (Ω_a), and carbonate (CO_3^{2-}) concentrations of seawater, which in turn, will depress coral calcification (Hoegh-Guldberg et al. 2007). Multiple studies have shown a strong and positive relationship between coral calcification and Ω_a (Langdon 2000), with declines in Ω_a depressing coral calcification by 20-40% for a doubling of pCO_2 from current levels (Hoegh-Guldberg 2005).

In addition to the interest in the consequences of OA for corals, the effects of rising temperature have also attracted attention, with the majority of this effort focusing on thermal bleaching (Brown 1997). Surprisingly however, few studies have simultaneously tested the effects of increases in temperature and declining pH (OA) on coral calcification, and in those studies that have, conflicting results have been obtained. For instance, one study found that high temperature and low pH acted in positive synergy to further depress coral calcification (Reynaud et al. 2003), while another reported that coral calcification under summer temperatures (27 ºC) was less affected by pH than under winter (23 ºC) temperatures (Langdon and Atkinson 2005). Potentially, some of the complexity

in the synergistic effects of temperature and OA on coral calcification is a result of the tight association between calcification and photosynthesis (McConnaughey and Whelan 1997), such that the effects of temperature and OA on calcification might not be independent of the effects on photosynthesis (Gattuso et al. 1999).

The objective of this study was to explore the interactive effects of high temperatures and high CO_2 on the calcification and photosynthesis of corals. We selected *Porites rus* and *Pocillopora meandrina* for this analysis because these species are common in the lagoon of Moorea, French Polynesia, where they form branching colonies that are tractable to manipulation. A microcosm system was used to expose the corals to either ≈27.3 ºC or ≈29.4 ºC at pH levels of 7.8 or 8.2, with the low temperature falling close to the ambient temperature (28.4 ºC) when the experiment was conducted (April 2007), and the high temperature being close to maximum temperature occurring in the lagoon (29.9 ºC); the two Ω_a levels represent present day conditions and those expected within ≈100 years (IPCC 2007).

Methods

Incubations were conducted for 2 weeks in April 2007 using a design in which corals were placed into one of four tanks (each 135 l) that created two crossed levels of temperature and Ω_a. The outcomes of the incubations were assessed through calcification and photosynthetic efficiency (maximum dark-adapted quantum yield, F_v/F_m), and were analyzed with a Model I, two way ANOVA. The statistical replicates were coral branches collected from *Porites rus* and *Pocillopora meandrina* growing at 1-2 m depth in the lagoon of Moorea. For each species, one branch (3.5–6 cm long) was collected from each of 44 colonies that were separated by 3-5 m to increase the likelihood that they were unique genotypes. Following collection, the fragments were transported to the lab and prepared as nubbins by attaching them to plastic bases with epoxy (Z-Spar A788). Nubbins were then placed in flowing seawater for 12 h and monitored visually for signs of damage. Healthy nubbins from each species were assigned randomly to one of the four incubation tanks, such that every tank contained equal numbers (n = 11) of *P. rus* and *P. meandrina*. Nubbins were acclimated to tank conditions for 24 h at a mean temperature of 28.4 ± 0.5 ºC (± SE, n = 54), a mean pH of 8.2 ± 0.1 (± SE, n = 76), and a constant mean light level of 480 ± 87 µmol photons m^{-2} s^{-1} (n = 12) provided with lamps (described below).

During treatments, nubbins were maintained in tanks located indoors, with each fitted with a chiller, heater, and pump that mixed and aerated the seawater. The seawater was replaced partially each day (20 % d^{-1}) with unfiltered seawater collected from the lagoon. Two 1000 W metal halide lamps (Sylvania BT37, Metalarc) were suspended 0.5 m above the tanks to provide light intensities of 797 ± 14 µmol photons m^{-2} s^{-1} (± SE, n = 148, measured with a Li-Cor LI 193SA) on a 12:12 light:dark cycle. The light intensity was selected to be less than the intensity at noon (1,720 ± 370 µmol photons m^{-2} s^{-1}, n = 17) at the collection depth when the experiment was conducted. Each tank was fitted with a microprocessor-controlled regulator (Neptune Systems) that operated the heaters, chillers, and pH regulation system to maintained temperatures with a resolution of ± 0.2 ºC, and pH with a resolution of ± 0.1 unit. Using this system, the two tanks at the control temperature were maintained at 27.2 ± 0.4 ºC and 27.5 ± 0.3 ºC (± SE, n = 236 and 246, respectively), and the two at the high temperature were maintained at 29.6 ± 0.2 ºC and 29.2 ± 0.4 ºC (mean ± SE, n = 290 and 249, respectively).

The pH of the tanks was controlled by adding dilute HCL or NaOH, using pumps operated by the regulators. The pH was logged using pH electrodes (Orion 9156 BN-WP, calibrated every 12 h using NBS buffers) attached to the regulators, and the output of the electrodes was used indirectly to operate the pumps adding acid or alkali. Using this system, the tanks at control pH were maintained at mean levels of 8.17 ± 0.01 (±SE, n > 278), and the treatment tanks were maintained at mean levels of 7.81 ± 0.05 (± SE, n = 587), and 7.82 ± 0.06 (± SE, n = 587). In addition to monitoring the pH, DIC parameters (Table 1) were assessed every 2-3 d by Gran titrations that were used to calculate total alkalinity (TA); TA and pH were used to calculate Ω_a using the CO2SYS program (Lewis and Wallis 1998) and the NBS buffer scale. The accuracy of the TA values was assessed using Certified Reference Materials (from Scripps Institute of Oceanography), which revealed downward discrepancies of 6% for TA and 11% for total DIC.

Calcification was assessed as the change in mass of the carbonate skeleton as determined by buoyant weighing, and was normalized to the coral area (µg mm^{-2} d^{-1}) determined by wax dipping (Stimson and Kinzie 1991). The maximum dark-adapted quantum yield of PSII (F_v/F_m) was measured before and after incubation for *Pocillopora meandrina* and *Porites rus* using a pulse-amplitude-modulation fluorometer (Diving PAM, Walz, GmbH) that was maintained at constant instrument settings for each species. Based on the results from previous studies, corals were dark adapted for ≥ 3 h prior to measuring F_v/F_m.

The results were analyzed using a two-way ANOVA, in which temperature and pH were fixed factors, and nubbins were statistical replicates. To

gain insight into the variation in calcification between each pH level within each temperature level (i.e., where a significant interaction was detected), a Student's t-test was subsequently applied, but as this represented an unplanned post hoc analysis, the results should be interpreted with caution. The statistical assumptions of normality and homoscedascity were tested through graphical analyses of the residuals, and all statistical tests were accomplished using JMP software (Version 7, SAS Institute Inc) running in a Windows environment.

Results

Of the 44 *Porites rus* nubbins used in the experiment, all appeared healthy throughout the incubations and, although slight paling did occur in all tanks, this was not associated with significant changes in F_v/F_m as compared to freshly collected corals ($p > 0.90$). *Pocillopora meandrina* appeared to be more sensitive to the incubation conditions, with some nubbins showed paling in the control and treatment for both pH and temperature conditions.

Calcification rates

For *Porites rus*, mass increased by 33 to 110 µg mm^{-2} over the 14 d incubation in all treatments (Fig. 1), and growth was affected significantly by a temperature x pH interaction ($F = 17.554$, df = 1,39, $p < 0.001$), as well as pH ($p < 0.001$), but not temperature ($p = 0.150$). At 27 °C, mean calcification rates were reduced 70% from 7.9 ± 0.8 µg mm^{-2} d^{-1} (± SE, n = 10) at pH 8.18, to 2.4 ± 0.7 µg mm^{-2} d^{-1} (± SE, n = 11) at pH 7.80, and this difference was significant ($t = 6.768$, df = 19, $p < 0.001$). At 29 °C, mean calcification rates were unaffected by pH ($t = -0.219$, df = 20, $p = 0.828$), and they changed only slightly from 6.0 ± 0.6 µg mm^{-2} d^{-1} (mean ± SE, n = 11) at pH 8.18, to 6.3 ± 0.9 µg mm^{-2} d^{-1} (mean ± SE, n = 11) at pH 7.80.

For *Pocillopora meandrina*, four nubbins (9% of the total) were removed from the analyses of calcification rate because they lost weight and displayed low values of F_v/F_m (< 0.500) that are indicative of poor health. Calcification rates for this species were also affected significantly by a temperature x pH interaction ($F = 4.819$, df = 1,36, $p = 0.034$), but neither of the main effects were significant alone ($p \geq 0.805$). At 27 °C, mean calcification rates were reduced 50% from 1.8 ± 0.3 µg mm^{-2} d^{-1} (± SE, n = 11) at pH 8.18, to 1.0 ± 0.1 µg mm^{-2} d^{-1} (± SE, n = 10) at pH 7.80, but this trend was not significant ($p > 0.05$). At 29 °C, the direction of the pH effect on calcification was reversed, with mean rates doubling from 1.0 ± 0.6 µg mm^{-2} d^{-1} (± SE, n = 9) at pH 8.18, to 2.0 ± 0.5 µg mm^{-2} d^{-1} (± SE, n = 11) at pH 7.80, although this trend was also not significant ($p > 0.05$).

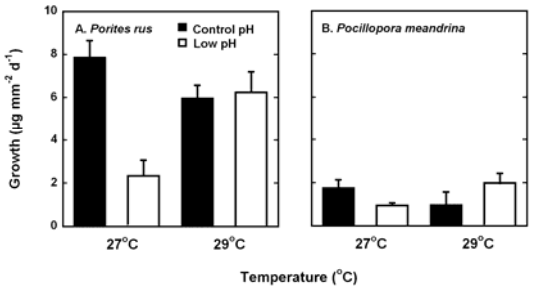

Figure 1. Effect of temperature and pH on the growth (µg mm^{-2} d^{-1}) of (A) *Porites rus,* and (B) *Pocillopora meandrina* during a 14 d incubation under combinations of control pH (8.2) and low pH (7.8); values shown are mean ± SE (n = 9-11 for each temperature and pH combination). Calcification was affected significantly by an interaction between temperature and pH in *P. rus* ($p < 0.001$) and *P. meandrina* ($p = 0.034$), largely because it was depressed by low pH at 27 °C, but not 29 °C.

Maximum dark-adapted quantum yield

The mean F_v/F_m values for all corals before treatment for *Porites rus* (0.573 ± 0.005, mean ± SE) and *Pocillopora meandrina* (0.631± 0.004, mean ± SE) were not significantly different ($p > 0.05$) from the average values in the control tanks after treatment (0.585 ± 0.012 and 0.640 ± 0.011, respectively, mean ± SE, Fig. 2), nor were any treatment combinations of temperature and pH significantly different from each other ($p > 0.05$). For *P. rus*, F_v/F_m was unaffected by pH ($F = 0.971$, df = 1,40, $p = 0.330$), and temperature ($F = 0.428$, df = 1,40, $p = 0.516$), and there was no interaction between the two ($F = 0.587$, df = 1,40, $p = 0.447$). A similar result was obtained for *P. meandrina,* with F_v/F_m again unaffected by pH ($F = 0.022$, df = 1,38, $p = 0.881$), temperature ($F = 0.278$, df = 1,38, $p = 0.600$), or the interaction between the two ($F = 1.108$, df = 1,38, $p = 0.299$).

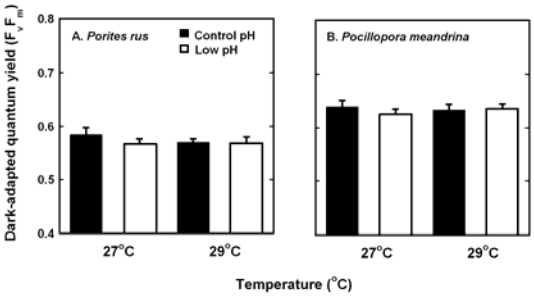

Figure 2. Maximum dark-adapted quantum yield of PSII (F_v/F_m) of (A) *Porites rus,* and (B) *Pocillopora meandrina* incubated for 14 d at control pH (8.2) and low pH (7.8); values shown are means ± SE, n=10-11 nubbins for each treatment combination. For both species, F_v/F_m was unaffected by pH ($p > 0.330$), temperature ($p > 0.516$), and the interaction between the two ($p > 0.299$).

Parameter	Control pH	Treatment pH
pH (NBS)	8.18 ± 0.01	7.80 ± 0.01
Alkalinity (μEquiv kg^{-1})	2143 ± 68	1638 ± 66
pCO_2 (μatm)	395 ± 40	720 ± 205
Total Carbon (μmol kg^{-1})	2010 ± 41	1692 ± 40
CO^{2-}_3 (μmol kg^{-1})	237 ± 11	126 ± 47
$\Omega_{aragonite}$	3.8 ± 0.2	2.0 ± 0.7
	n=8	n=6

Table 1. Characteristics of the seawater in the four tanks over the 14 d experiment; all values are means ± SE.

Discussion

The effects of high temperature and OA on coral reefs are predicted to compromise calcium carbonate accretion, reduce coral diversity, and weaken the carbonate reef framework (Hoegh-Guldberg 2005). The likelihood of such outcomes is increased by the lack of evidence demonstrating that corals can acclimatize to the effects of OA (Langdon et al. 2000). Nevertheless, there is good reason to suppose that corals exhibit the same kinds of abilities to acclimatize as most eukaryotes (Gates and Edmunds 1999), and for rising temperature there is evidence that some corals can acclimatize to warmer conditions (Jones et al. 2008). Thus while there is a consensus regarding the negative implications for corals of increasing CO_2 and rising temperature (Hoegh-Guldberg et al. 2007), at least when the factors act in isolation, there is less certainty over their interactive effects (Kleypas and Langdon 2006). In order to make accurate predictions of the responses of coral reefs to GCC, experimental manipulations on short time scales are required to understand the potential for interactive effects of CO_2 and temperature.

Utilizing mesocosms, the present study examined the effect of OA on calcification and photosynthesis of *Porites rus* and *Pocillopora meandrina* under two different temperature regimes predicted to occur in the next 100 years (IPCC 2007). An interesting outcome of these analyses is the demonstration that a decrease in pH interacts with small differences in temperature to decrease growth at 27.3 ^{o}C, but not at 29.4 ^{o}C; this effect was striking for *P. rus*, but was less developed in *P. meandrina*. These finding differ from those of Reynaud et al. (2003), who found that the inhibitory effect of elevated CO_2 (760 ppm versus 460 ppm) on the growth of *Stylophora pistillata* were accentuated at higher temperature (28 ºC versus 25 ºC). However, Reynaud et al. (2003) also found no inhibitory effect of high CO_2 at 25 ºC, which is inconsistent with previous studies that have reported inhibitory effects of high CO_2 on coral calcification over a range of temperatures (23 - 27 ºC) (Kleypas and Langdon 2006). However, Langdon and Atkinson (2005) reported findings that are similar to the present study, principally by showing indirectly that higher temperature may alleviate the effects of increased CO_2 on coral calcification. Importantly, they found that a reduction in pH of 0.22-0.28 depressed the calcification of *Montipora capitata* and *Porites compressa* by ≈80% during the cool winter, but by only ≈40% in the warm summer, although the net effect of temperature on the relationship between Ω_a and calcification was not significant (Langdon and Atkinson 2005). The present findings could have biological significance as they suggest that elevated temperature can mitigate the effects of rising CO_2, at least for some corals over a short period.

In the context of evaluating how temperature might alleviate the effects of high CO_2 on coral calcification, it is noteworthy that temperature has numerous biological effects that operate in a biphasic pattern, with rising temperature stimulating processes to a threshold value, and inhibiting them beyond this point. For corals, rising temperatures stimulate enzyme activity (Marshall and Clode 2004) and metabolic rate (Edmunds 2005), and drives the classic bell-shaped relationship between calcification and this physical factor (Marshall and Clode 2004) with typical threshold temperatures of 26-28 ºC (Buddemeier and Kinzie 1976). Together, these results suggest that the temperature manipulations used in this study might not have surpassed the threshold value for calcification in *Porites rus* and *Pocillopora meandrina* in the warm lagoon of Moorea, perhaps because acclimatization to local conditions (Jones et al. 2008) has lead to higher threshold temperatures in this location. With a high threshold temperature for calcification (e.g., > 29 ºC), the effects of the temperature increase in the present study may have been sufficient to offset the effects of carbonate limitation caused by low pH, at least temporarily.

The lack of a significant effect of temperature and pH on F_v/F_m indicates that the high temperature treatment (29 ºC) in this experiment did not reduce photosynthetic efficiency in photosystem II (PSII) when compared to the control temperature treatment (27 ºC). Although temperatures above 29 ºC can reduce photosynthesis in hermatypic corals (Coles and Jokiel 1977), there is considerable evidence demonstrating that the severity of thermal stress is best measured by its magnitude relative to the local seasonal maximum temperature (Jones et al. 2008), with increases of 1 to 2 ºC above this value typically serving as reliable predictors of photosynthetic damage (Hoegh-Guldberg et al. 2007). In the lagoon of Moorea, the maximum temperature recorded at the site of collection is 29-30 ºC, and therefore the high temperature used in the present study may have been too low to illicit a negative response in photosynthesis in *P. rus* and *P. meandrina*. In this context, the

absence of a pH effect might be construed as preliminary evidence that more acidic conditions do not enhance the sensitivity of F_v/F_m to high temperature.

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Remote sensing of changes in carbonate production on coral reefs: The Florida Keys

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Abstract. Using satellite remote sensing, it is possible to scale-up *in situ* carbonate production observations on coral reefs from habitat (10^{-1}-10^{0} km^2) to regional (10^{3}-10^{4} km^2) scales. Using a time series of Landsats 5 TM and 7 ETM+ spanning 18 years from 1984-2002 with 30 m spatial resolution, it is possible to quantify changes in the spatial extent of reef habitats in the Florida Keys. This study focused on the shallow (<6 m) backreefs, reef flats, and forereefs along the length of the Florida Keys. Twenty-eight georectified images were subjected to atmospheric and water-column corrections, then calibrated for change detection analyses. Published values for production were applied additively to estimate overall changes in production. A reduction in cover of coral habitat of >50% is observed both *in situ* and by satellite data over this time, and is reflected in an estimated decline in production of ~39%. Similar approaches could be applied over other areas with good temporal availability of remote sensing imagery validated by *in situ* observations. Using remote sensing as a tool for scaling-up *in situ* measurements of production has potential applications for modeling synoptic scale environmental impacts, such as the broad reaching effects from ocean acidification.

Key words: remote sensing, ocean acidification, carbonate, Landsat, change detection.

Introduction

Mounting evidence suggests that global climate change, including ocean acidification, will have negative impacts on marine calcifiers, especially coral reef ecosystems (Smith and Buddemeier 1992; Kleypas et al. 2001; Walther et al. 2002; Hughes et al. 2003; Kleypas et al. 2006; Kuffner et al. 2007). Increases in atmospheric and oceanic partial pressure of carbon dioxide (pCO_2) drive changes in the carbonate chemistry of surface ocean waters, decreasing pH and carbonate ion concentration [CO_3^{-2}] (Zeebe and Wolf-Gladrow 2003; Feely et al. 2004). The drop in pH and [CO_3^{-2}] decrease the aragonite ($CaCO_3$) saturation state, making it more difficult for corals to secrete their aragonite skeletons (Gattuso et al. 1998; Kleypas et al. 1999; Gattuso and Buddemeier 2000; Fine and Tchernov 2007).

However, some *in situ* research has indicated that on the scale of reefs or reef biotopes, the net production of carbonate can vary widely (Yates and Halley 2006). Therefore, neither simple geochemical extrapolations, nor single reef-scale metabolic estimates alone can be reliably used to monitor the impact of environmental changes such as ocean acidification on reef processes across time without accounting for changes in reef communities and habitats.

The net metabolism of a given reef zone or biotope can be accounted for by the balance of productivity, or gross photosynthetic carbon fixation (*P*), and respiration (*R*), or their difference, the measure of excess production (*E*) (Kinsey 1985). In addition to carbon metabolism, the amount of carbonate mineral precipitation (*G*) can also be an estimate of reef "health" (Kinsey 1985).

Kinsey (1983) established a linear range of reef metabolic end members, with *P* ranging from 1 g C m^{-2} d^{-1} (100% sand and rubble) to 20 g C m^{-2} d^{-1} (100% coral-algal hard substrate). Calcification also had a linear relationship to the same end members with *G* ranging (respectively) from 0.5 g $CaCO_3$ m^{-2} d^{-1} to 10 g $CaCO_3$ m^{-2} d^{-1} (Kinsey 1983). These metabolic parameters are assumed to have additive properties when scaling-up with remote sensing (Atkinson and Grigg 1984; Andréfouët and Payri 2001).

Despite the general agreement on reefs as sources of CO_2, there is evidence that certain reefs, or parts of reefs, are sinks for atmospheric CO_2 (Kayanne et al. 1995; Ikeda et al. 1997; Yates and Halley 2006). Additionally, recent direct measurements on Moloka'i (Hawai'i, USA) indicate that a reef may operate simultaneously as both a source and a sink in patchy patterns related to biotope spatial distribution (Yates and Halley 2006). This habitat-scale spatial variability of CO_2 production across reefs reflects similar patchiness in carbonate precipitation.

Here we: (1) demonstrate the potential of a time series of Landsat imagery to estimate changes in reef carbonate production over large areas, and (2) report that Landsat based estimates indicate a ~39% reduction in carbonate production across the scope of the Florida Keys since 1984. These results provide important estimates of carbonate precipitation and ecosystem functioning over spatial and time scales that are relevant to ecosystem based management and models of regional responses to ocean acidification.

Material and Methods

Location

The Florida Keys are a chain of low islands extending nearly 400 km from the southeastern tip of Florida to the south and west forming the seaward margin of Florida Bay. In the Keys, the basic zonation proceeds seaward from the islands with a broad, shallow seagrass flat, followed by Hawk Channel with seagrass and patch reefs, then shallowing again to the barrier reef complex ~5 km offshore. The Florida Keys have an unfortunately well documented record of decline in reef health in recent decades (Dustan et al. 2001; Gardner et al. 2003; CREMP 2005; Palandro et al. 2008). This study focused on several shallow portions of the barrier reef complex ≤6 m deep, specifically at Carysfort Reef, Grecian Rocks, Molasses Reef, Looe Key Reef, Western Sambo, and Sand Key Reef (Fig. 1). These sites are representative of shallow barrier reefs of the Florida Keys. They were selected in part because each is a Sanctuary Preservation Area within the Florida Keys National Marine Sanctuary, and in part because these sites have been monitored annually by the Coral Reef Evaluation and Monitoring Program (CREMP) since 1996.

Image Classification, Validation, and Interpretation

Eight Landsat 5 TM and four Landsat 7 ETM+ images were used for this study. Both satellite sensors are equipped with three discrete visible spectral bands useful for this study, specifically the blue (450 nm–520 nm), green (520 nm–600 nm) and red (630 nm–690 nm) bands. This study used images acquired in 1984, 1988, 1992, 1996, 2000, and 2002 during the spring season (March–May). Two Landsat scenes were required to cover the Florida Keys Reef Tract (path/row 15/43 and 16/43). The images were resampled at 30 m spatial resolution, georectified, and transformed to at sensor radiance (L) using calibration coefficients provided with each file. Atmospheric and water-column corrections were applied to each image, and images were calibrated for change detection by an empirical line calibration as detailed in Palandro et al. (2008). Detailed image classifications for these sites are also available in Palandro et al (2008).

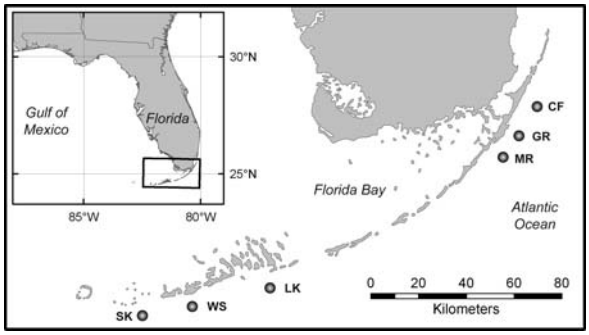

Figure 1. Location map of study sites in the Florida Keys. CF = Carysfort Reef; GR = Grecian Rocks; MR = Molasses Reef; LK = Looe Key Reef; WS = Western Sambo; SK = Sand Key Reef.

From *in situ* observation, four benthic biotopes (i.e., classes) were determined: sand, sparse live substrate, dense live substrate, and coral habitat. Dense live substrate, as measured on Florida patch reefs by Brock et al., (2006), consisted of an average of ~7% live hermatypic coral cover, ~27% macroalgae (calcareous and fleshy), about 10% gorgonians, and 21% bare hardbottom. Sparse live substrate represents areas with the same components as dense live substrate, but with ≥70% bare hardbottom. The sand class is dominated by unconsolidated carbonate sands of varying thickness. The coral habitat biotope represents areas with *in situ* observations of 22% live hermatypic (scleractinian plus hydrocoral) coral cover.

Image classification is based on training pixels determined from *in situ* observations in the same period as the 2002 Landsat images. Image classification was performed using a supervised Mahalanobis Distance classifier with ENVI image software (v4.3). Additional validation of classification was performed by proxy using higher spatial resolution IKONOS imagery, and by *in situ* data compiled from published (Lang 2003; CREMP 2005) and unpublished sources in that time interval.

For this study, the transformed divergence class separability statistic indicated each class was statistically distinct with a mean TD=1.91. Unfortunately, rigorous accuracy assessment is impossible to apply to historical data that were not collected in the context of a remote sensing study with image-driven sampling protocols. In this study,

accuracy assessment of the classification for historical images was performed by generalized comparison to CREMP data.

Carbonate Parameters and Scaling

This project takes advantage of values for reef carbonate production recently reported by Brock et al. (2006) for similar biotopes, but over a smaller area of the North Florida Reef Tract (Tab. 1). The values for *G* were measured with a unique *in situ* mesocosm, the Submersible Habitat for Analyzing Reef Quality (SHARQ) that is operated by the U.S. Geological Survey (Yates and Halley 2003). Brock et al. (2006) presented values for *P*, *R*, *P/R*, *E*, and *G* for three reef biotopes common to our sites: sand, dense live substrate, and sparse live substrate. For this current study, we adopted those biotope definitions and carbonate production values. The coral habitat class value for *G* is calculated here as a linear mix of calcification end members (Kinsey 1983) representing 22% live coral cover (Tab. 1). With no *in situ* SHARQ data available for a class with 22% live coral cover, the Kinsey (1983) end members for *G* offer a suitable basis for interpolation.

Table 1. Values used for estimation of carbonate production. * = directly from Brock et al. (2006).

Class	*G* (g $CaCO_3$ m^{-2} d^{-1})
Sand*	0.11
Sparse Live Substrate*	-0.04
Dense Live Substrate*	1.29
Coral Habitat	2.64

Carbonate precipitation on coral reefs is assumed to be an additive process such that the whole is equal to the sum of the parts (Atkinson and Grigg 1984; Hatcher 1997). For example, the contribution of the coral habitat class to the carbonate production (*G*) for the whole of a given reef complex is a function of the relative fractional area (m^2). The total carbonate production for a reef area depends on the sum of the production of the included biotopes (sand, dense live substrate, sparse live substrate, coral habitat). The measured value for *G* (g $CaCO_3$ m^{-2} d^{-1}) for each biotope (Tab. 1) is multiplied by the fractional area covered by that biotope ($F_{biotope}$). It follows as Eqn. 1:

$$G_{reef} = (F_{sand} \times G_{sand}) + (F_{dense\ live} \times G_{dense\ live}) + (F_{sparse\ live} \times G_{sparse\ live}) + (F_{coral\ habitat} \times G_{coral\ habitat}) \quad (1)$$

Two values for G_{reef} were calculated at each time step. The more conservative number was generated by assuming that in 1984, areas classified as coral habitat, consisted of 22% live coral cover like they do today, thus assuming modern levels of production (*G*) as well. However, this is not likely given the known declines in percent coral cover (Gardner et al. 2003; CREMP 2005). Integration of trends from published (Dustan 1985; CREMP 2005) and unpublished data for these sites produced a simple model of hindcast percent live coral cover at each site during each time interval. The model was based a linear extension of trends from CREMP data (CREMP 2005) at each of the sites, validated where possible by comparison with observations of coral cover during the 1980's. The hindcast live coral cover values in 1984 ranged from 38%-56% which was reflected in corresponding hindcast estimates of carbonate production (G_H).

Results

Validation of the benthic biotopes demonstrated that classification of the most recent (2002) Landsat images was 86% (±2%) accurate across the six reefs in this study, similar to results over much broader regions of the North Florida Reef Tract (Moses et al. in press). Sand has a broad spectral signature, leading to the most frequent misclassification of these four biotopes, typically misclassified as sparse live substrate. However, misclassification of sand as sparse live substrate has a relatively small impact on estimates of G_{reef} compared to changes in percentage of coral habitat, due to the relatively small values of *G* in those biotopes.

The decline of coral habitat correlates significantly (r^2 = 0.71, N = 32) with the decline in percent live coral reported by CREMP. It is important to point out that the decline measured by the 30 m Landsat pixels is decline in the area of coral habitat (a biotope that averages 22% live cover), and is not the same as the decline in percent coral cover as detected by CREMP. However, the two measures are directly related (Palandro et al. 2003; Palandro et al. 2008).

The mean conservative change in carbonate production (ΔG) between 1984-2002 over the six reefs was -20% (±22%). Without hindcast adjustment, Sand Key Reef even appears to slightly increase *G* over the study period. The mean hindcast ΔG_H suggests a greater decline of -39% (±14%) (Fig. 2). The more realistic hindcast values ranged from ΔG_H = -15% at Sand Key Reef to ΔG_H = -57% at Western Sambo.

During the period from 1996-2002, all sites suffered a steady decrease in the coral habitat biotope. Despite this, four of the six sites actually suggest slight increases in carbonate production in the 2000 or 2002 estimates. In the case at Grecian Rocks between 1996-2002, area percent of coral habitat biotope decreased from 21.7% to 14.0%, yet carbonate production increased from 1.13 to 1.20 g $CaCO_3$ m^{-2} d^{-1}.

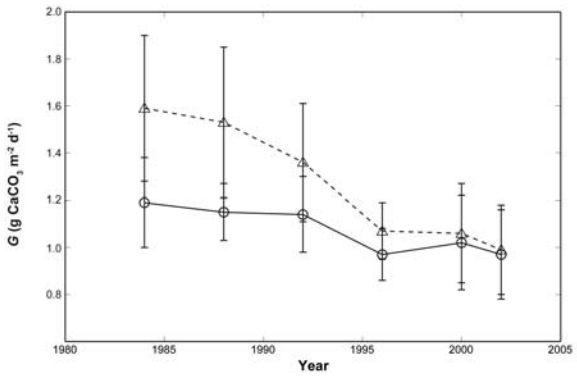

Figure 2. Average satellite estimated decline in carbonate production in the Florida Keys. Solid line with circles represents estimations based on a consistent 22% live cover (G). Dashed line with triangles represents estimates based on hindcast live coral cover (G_H).

Discussion

Work presented here suggests substantial declines in carbonate production on reefs in the Florida Keys. The roughly 40% decline in carbonate production over about two decades, an average rate of decrease of ~2% per year, implies a substantial limitation from broad reaching factors on reef accumulation in the Florida Keys.

The observed increases in G estimated for 2000 and 2002 for some sites are likely an ecological recovery in response to the 1998 bleaching event. The 1998 bleaching event reduced the actual percent live coral cover (CREMP 2005), transforming coral habitat area to sparse live substrate. Subsequent recovery of the sparse live substrate to dense live substrate increased the carbonate production, but not the area coverage of coral habitat, which continued to decline. Unfortunately, trends of live hermatypic coral cover on Florida reefs continue to suffer under substantial stressors (Maliao et al. 2008), making recovery of dense live substrate to coral habitat seem unlikely.

These results do not specifically confirm direct influence of ocean acidification on carbonate production and reef accretion in the study area. However, the results describe the magnitude and geographic scope of decline in carbonate mineral precipitation in the Florida Keys, which likely stems from a combination of antagonistic environmental factors operating across a range of scales (Gardner et al. 2003; CREMP 2005).

Models like this have the potential to be accurately applied over regions with good *in situ* habitat cover data as well as carbonate production observations. The additive model reported here does not account for complexities such as sea surface temperature or water retention time. However, improved estimates can be made by incorporating more environmental data that could influence the calculations of G. Inclusion of such components would result in a gradient of carbonate production values in each model grid cell (i.e., Landsat pixel). This would be more realistic than the current list of discrete values for G. Additional *in situ* data collection, with systems like the SHARQ, will provide more control points for benthic community production of carbonate, especially if measurements are expanded into deeper waters of previously unsampled geographic areas.

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Fine banding in the septa of corals.

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Abstract. Fine banding is apparent in the crystalline areas of thin ground and polished sections of coral septa when viewed with phase contrast optics. In some septa (e.g. *Meandrina meandrites*) the banding is robust and a series of laminations representing several months of growth can be followed. Repeated staining with alizarin of small colonies of *Agaricia* confirmed that the bands are diurnal and indicated that the darker bands (optically more dense) are formed during the day and the lighter bands at night. Ground and stained sections of *A. agaricites* mounted in epoxy resin showed, particularly in the calicoblastic layer areas with high calcification, many bunches of spherical bodies (Golgi apparatus?). These are probably involved in the secretion of the skeletal matrix. Evidence is presented that the Ca^{2+}ATPase pump may be light sensitive and leakage of Ca^{2+} due to lipid peroxidation of the plasma membranes during the day accounts for the higher calcification rates in light. A model of calcification is presented suggesting a mechanism by which the diurnal banding is produced.

Key words: Coral calcification, light, diurnal banding, Ca^{2+}ATPase pump

Introduction

Fine banding in coral skeletons has been studied with a variety of techniques (Risk and Pearce 1992; Perrin 2003; Gill et al 2006). Daily or circadian banding has been reported in the epitheca of corals but is of limited use in environmental studies (Risk and Pearce 1992). Some microbands appear to be the result of secondary skeletonization processes or later infilling of pore spaces between the theca. (Gill et al 2006). Septa, however, have received little attention. They can be easily removed from live corals and, if fine banding is present and can be confirmed as diurnal, septa have the potential to provide a record of recent environmental events on a daily time scale. In addition to providing growth rate information there is the potential of providing chemical proxies which can be related to environmental variables in the form of trace elements and stable isotopes using laser ablation techniques. The aim in this study was to determine whether coral septa have fine diurnal bands and explore how light may be involved in their formation.

Materials and methods

All sea water used for experiments was passed through a column of 4-8 mesh activated charcoal (Sigma) and millepore-filtered (0.45µ). Small pieces of live coral were collected in the back reef at Discovery Bay, Jamaica and were held in the sea water table at the Discovery Bay Marine Laboratory of the University of the West Indies. Small platelike colonies of *Agaricia agaricites* with a thin outer edge proved to be a particularly useful source of material as the structure was simple, consisting of parallel septa, without polyps. Pieces could be easily trimmed to a desired shape with small scissors.

For alizarin staining small colonies of Agaricia were collected from the back reef and at 0900 hrs placed in a large clear plastic bag with 20mg/l of alizarin red. The colonies were removed at 1800 hrs to the water table where they were held in running sea water. Four days later they were again placed in sea water with stain, at midday, and returned to the water table at 1800 hrs. Forty eight hours later the corals were water-piked clean and dried.

Small pieces of *Agaricia* were fixed in modified Karnovsky solution (2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M Sorenson buffer), dehydrated, embedded in LR White (hard) acrylic resin (Sigma) and cured at 60ºC under nitrogen. Slices were cut with a diamond saw. The slices, mounted on glass slides with thermoplastic cement, were ground with a series of microabrasive sheets to a thickness of less than 0.05 mm. The sections were then ground and polished on one side, heated, detached and turned over. After re-attachment they were ground and polished on the second side and stained with toluidine blue. After washing in distilled water and drying the sections each received a drop of immersion oil and a cover glass.

Septa were carefully removed, oriented and mounted on glass slides with thermoplastic cement (Crystal Bond). For cross sections larger septa were first sectioned with a diamond saw, ground with a series of microabrasive sheets, polished on one side,

detached on a hot plate, turned over, reattached and ground and polished on the other side. Progress was monitored regularly under a microscope (Wild M20 provided with phase contrast optics) and turned over several times during grinding to ensure that the final result was a section through the centre of a trabecula. Photographs were taken with an Epson PC800 digital camera.

The acetoxymethyl (AM) ester of the cell permeant calcium indicator calcium orange (Molecular Probes Inc.) is permeable to living cells. On entry it is hydrolysed to release the fluorescent calcium indicator in a charged form that is less permeant to cell membranes. 50 µg of calcium orange was dissolved in 0.1ml of a 20% solution of Pluronic® F-127 in dimethyl sulfoxide (DMSO). This was added to 20 ml of sea water to give a 4 µM working solution. Small pieces from the edge of thin plates of *Agaricia agaricites* were incubated for 30 min, rinsed in sea water, trimmed and placed with 0.6 ml sea water in a sample vial in the fluorometer (Sandeman 2006). The sample vial was a 9x30 mm Kimble glass vial with a neoprene 0-ring round the top and the bottom was shielded with a disc of black electrical tape. Excitation at 532 nm was provided by a green laser diode module (E1894, Egismos Technology Corporation). The beam was spread by a double concave lens in its path. Fluorescence (peak at 575-580 nm) collected by the ellipsoidal mirror is passed through a broadband interference filter (560-640 nm, Edmund Optics, A46-159) to the 2.0x2.0 cm blue enhanced silicon photodiode, PerkinsElmer Optoelectronics, at the second conjugate focus of the mirror. In the lamp housing three miniature blue light emitting diodes (LEDs, Maplin Electronics, N31AT) arranged round the excitation beam provided continuous illumination to the sample. The LEDs were independently switched and each provided approximately 100 µmol.m-2.s-1 at the bottom of the sample vial as measured with a LI-COR Quantum Radiometer (Model LI-250). The supply voltages for the laser diode and LEDs were regulated. For fluorescence readings the laser was switched on for as short a time as possible. Fluorescence usually decreased briefly after being switched on but quickly stabilized. Preliminary experiments indicated that photo-bleaching did not occur and fluorescence from coral or algal pigments did not interfere with the signal from the intracellular calcium. There was only slight leakage of the calcium probe.

Results

Of the species investigated *Meandrina meandrites* had robust easily seen banding (Fig. 1a-b). Each septum consistes of a palisade of parallel trabeculae with sclerodermites, or whorls of fibres, radiating out from growth centres. In longitudinal section the banding is continuous across two or more trabeculae (Fig. 2b). In vertical sections (Fig. 2a) the series of bands

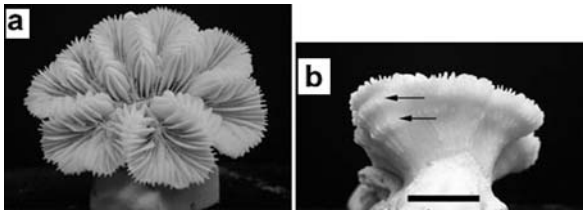

Figure 1a-b: Skeleton of colony of *Meandrina meandrites*, scale bar 1 cm. a septa. b epitheca with summer growth ridges(arrows).

can be followed for a considerable distance and the variation in the separation of the bands is considerable (4-15µ).

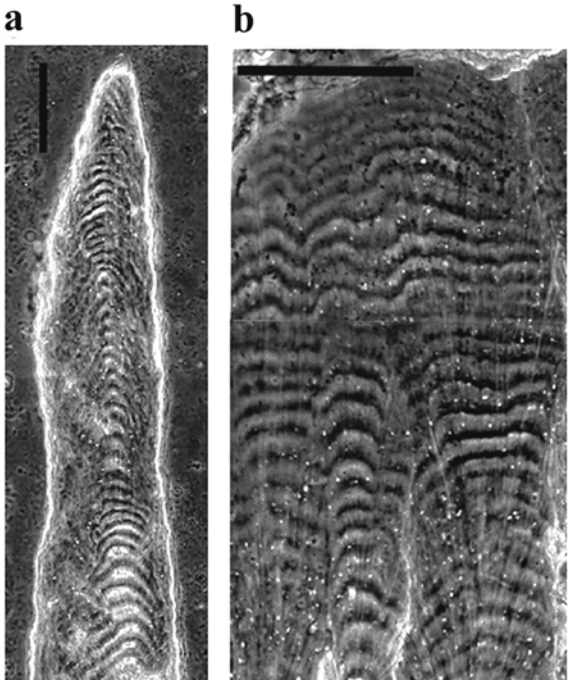

Figure 2a-b: Ground and polished thin sections of septa from *Meandrina meandrites* viewed with phase contrast optics, Scale Bar 100µm. a Vertical section, b Longitudinal section.

A similar arrangement of the trabeculae and banding was seen in several other coral species investigated: Dendrogyra cylindricus, *Dichocoena stokesii* and *Eusmilia fastigiata*. In *Montastrea annularis* (Fig. 3a) the trabeculae and pattern of banding were only visible in patches and sometimes obscured by whorls of radiating fibres or sclerodermites. In *Siderastrea siderea* central strips of crystalline banding were surrounded by amorphous material (Fig. 3b) In some species, especially those with thicker septa, eg *Dendrogyra cylindricus*, the trabeculae are not parallel and have a three dimensional fanlike arrangement

which makes the banding more difficult to follow. Some of the faster growing shallow water corals examined, *Acropora cervicornis, Siderastrea radians* and *Porites porites* appeared to have few crystalline areas with visible banding and most of the skeleton appeared to be deposited in an amorphous form.

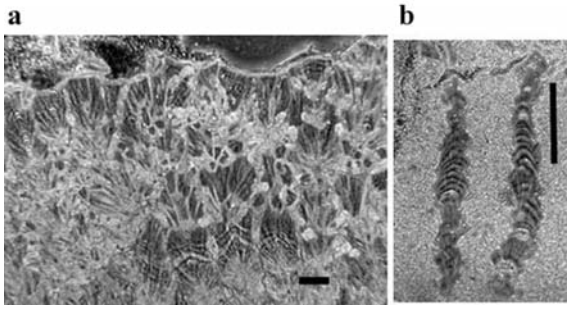

Figure 3a-b: Ground and polished thin sections of septa viewed with phase contrast optics, Scale bar 50µ. a *Montastrea annularis*. b *Siderastrea siderea***.**

The epitheca (Fig. 1b) of the colony of *Meandrina meandrites* from which the septa shown in Fig. 2 were taken had annual protruding ridges 4 mm apart. This translates into an average daily growth rate of 11µ which fits well with the separation of the laminations of the septa. Sections from the small colony of *Agaricia agaricites* which had been stained twice with alizarin four days apart (Fig. 4) confirmed that the laminations are diurnal and indicated that calcium was laid down during the day in the darker bands.

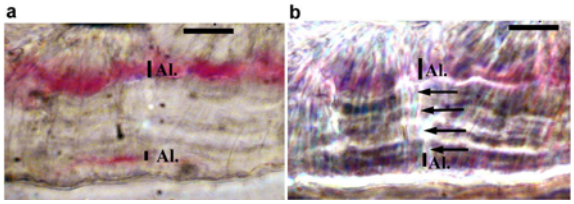

Fig. 4**:** Ground and polished sections of a septum of ***Agaricia agaricites*** after double staining. Scale bar 10µ. Al indicates stained layers, arrows dark bands. a standard optics. b phase contrast.

The two layers lining the skeleton of *Agaricia agaricites* can be distinguished in section (Fig.5). Zooxanthellae were not seen in the inner endodermal layer and both layers contain many clumps of vesicles that stain with toluidine blue indicating that they probably contain protein. These clumps of vesicles were profuse where calcification, as judged by the take-up of alizarin by the skeleton, was strongest, particularly on the septa. The stained vesicles were not seen in other tissues and it seems likely from their position and the quantity of material they contain that the vesicles could be the source of the organic matrix.

Fluorescence levels from pieces of *Agaricia* loaded with calcium orange leveled off within 10-15 minutes (Fig.6). The variation in fluorescence levels between different pieces of ***Ag****aricia* was high and depended on the extent to which the fluorescent probe was taken up by the coral pieces. When illuminated continuously with blue light from one or more LEDs the fluorescence level dropped quickly and adjusted to a new level. When the LEDs were switched off fluorescence returned slowly to close to its original level.

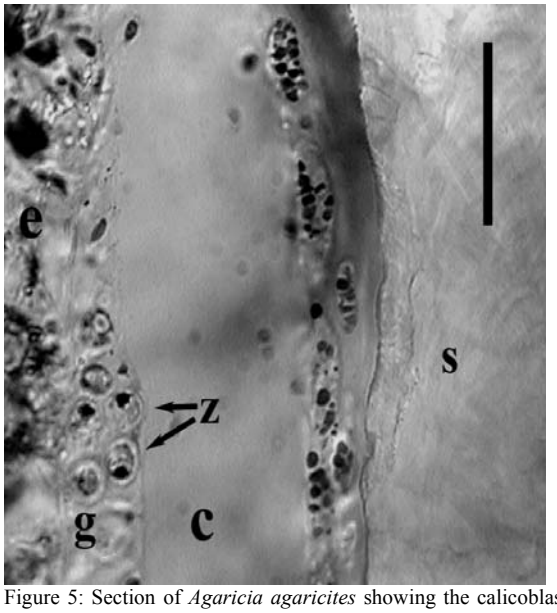

Figure 5: Section of *Agaricia agaricites* showing the calicoblastic endodermal and ectodermal layers, detached from the septal wall, with cells with stained secretory vesicles. **c** coelenteron, **e** epidermis, **g** gastrodermis, **s** septum, **z** zooxanthellae, Scale bar 50 µm.

Fig. 6 shows a typical result obtained using the same piece of *Agaricia* exposed to three different light levels with a dark period. As can be seen the change in fluorescence was proportional to the light intensity. With two LEDs switched on (200µmols.m^{-2}.s^{-1}) the fluorescence dropped to a new mean level 33.5 % lower than the original level (n=7).

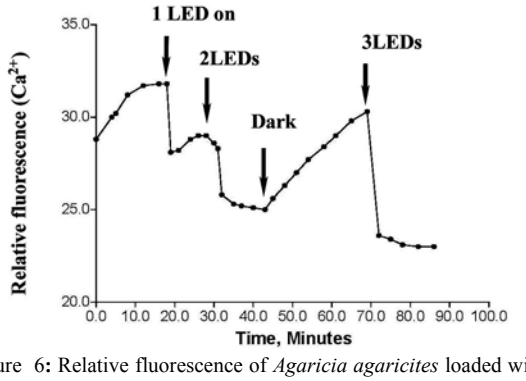

Figure 6**:** Relative fluorescence of *Agaricia agaricites* loaded with the intracellular fluorescent probe calcium orange. The relative change of fluorescence with time is shown for changes in light level (each blue LED emits 100 µmol.m-2.s-1).

Discussion

Comparison of the average separation of the bands of *Meandrina meandrites* (Fig. 2) with the annual ridges on the epitheca (Fig. 1b) and the sequential alizarin

staining of *Agaricia agaricites* (Fig. 4) indicated that the banding is diurnal. From the septa of the corals examined it is clear that a few species have the potential to provide a useful daily record of growth over periods of several months. These are generally species in with large, thin septa with robust banding in which the trabeculae are parallel and growth takes place in a single plane, eg *Meandrina meandrites*, *Fungia* sp. and *Eusmilia fastigiata*. In species such as *Dendrogyra cylindricus* with thick septa, many trabeculae grow at an angle to the main axis and these thicken the septae. However, this makes it difficult to find and follow the main growth axis. In other species the banding is either too patchy to follow or there are deposits of amorphous material with little or no banding visible. Variation in the separation of the bands in the septae of *Meandrina* was from 4-15 μ and bands can be counted up to the surface so that the exact date of variations can be pinpointed accurately.

Cohen and McConnaughey (2003) suggested that the role of the vesicles in the calicoblastic epithelium was the transport of seawater into the calcifying space. The clumps of vesicles seen in the two layers lining the skeleton in this study look like Golgi bodies which are often associated with the secretion of glycoproteins. The vesicles take up stain and are therefore unlikely to contain seawater and are interpreted here as the probable source of the organic matrix. The volume occupied by the vesicles (Fig. 5) appears to be enough to provide the 3-4 μ layer that would be laid down daily. No zooxanthellae were seen in the inner 'endodermal' layer and the two layers appeared very similar and vesicles were present equally in both layers, indicating that both layers may be involved in skeletogenesis. Physical extension of the coral takes place at night (Vago et al 1997) and this is presumably when the organic matrix is secreted. The role of the matrix is reviewed by Cohen and McConnaughy (2003). The matrix has been shown by Tambutté *et al* (2007) to consist of structural proteins which also play a catalytic role similar to that of carbonic anhydrase. It has also been shown to have calcium binding properties (Isa and Okazaki 1987; Constantz and Weiner 1988; Puverel et al. 2005). The dynamics of the synthesis and deposition of the organic matrix is not well known (Allemand et al, 1998) but its production may be the determining factor for calcium deposition rather than Ca^{2+} (Wainwright, 1963; Allemand et al 1998). The observation that the concentration of vesicles is highest in the areas of the coral where growth is highest may confirm this view.

Corals, like other organisms, maintain low intracellular Ca^{2+} levels (Barnes and Chalker 1990; Al-Horani et al, 2003) and failure of the Ca^{2+}ATPase pump at high temperatures was involved in 'dark' bleaching (Sandeman 2006). 'Solar' bleaching involved build-up of H_2O_2 produced in photosynthesis. H_2O_2 at lower levels may also play a role in the regulation of algal density (Sandeman 2006). It is also evident, from the stable isotope fractionation of calcium (Gussone et al 2005; Bohm et al 2006), that the Ca^{2+}ATPase pump is involved in transporting Ca^{2+} into the extracellular calcifying fluid (ECF) where it is deposited. Many mechanisms have been invoked to explain the significantly higher calcification rates of corals in light than in the dark, these were reviewed by Gattuso et al (1999). More recently Al-Horani et al (2003), using Ca^{2+} microsensors found that the Ca^{2+} concentration under the calicoblastic layer of *Galaxea fascicularis* increased in the light and when the light was switched off the Ca^{2+} decreased. This was interpreted as calcium transport to the skeleton site being triggered by light with the additional ATP needed being supply from photosynthetically driven respiration. Cohen and McConnaughy (2003) proposed a model of calcification based on this light-sensitive action of the Ca^{2+}ATPase pump. The experiments undertaken in this study with *Agaricia agaricites* loaded with the fluorescent intracellular calcium probe indicated that the intracellular Ca^{2+} concentration decreases rapidly by 33% in light (200 μmol photons m^2. s^{-1}). The decrease was more or less proportional to the light intensity. In the dark the Ca^{2+} concentration returned slowly to its previous level. These results are interpreted as confirmation that the Ca^{2+}ATPase pump is light sensitive. However increased activity of the Ca^{2+}ATPase pump in the light does not explain how Ca^{2+}, which because of its charge is unable to move passively through the plasma membrane, gets into the calicoblastic layer in the first place from the large pool (Goreau 1959) in the coelenteron. Mechanisms involving special channels (Zoccola et al 1999; Clode and Marshall 2002) or special cells (Tambutté et al 1996) or transport of seawater by pericellular pathways or vesicles (see Cohen and McConnaughey 2003) have been suggested. Sandeman (2008) showed that lipid peroxidation in *Agaricia agaricites* in light (800μ mol photons) increased to a maximum in about four hours then reduced back to its original level. Lipid peroxidation, produced as a result of build up of H_2O_2 is known to make membranes leaky to Ca^{2+} (Halliwell and Gutteridge 1999) and it was suggested that the leakage provides a route for additional Ca^{2+} and other ions to enter cells of the calicoblastic layer. In that study the build-up of lipid-peroxidation parallels, almost exactly, the way that calcification rates of *Galaxea fascicularis* in light (200 μmol photons) measured by al-Horani (2007) increased to a maximum in six hours then decreased again. The difference in timing can be attributed to difference in light levels. Lipid peroxidation levels at the beginning of experi-

ments (Sandeman 2008), before exposure to light, and at the end of experiments were about 50% of the highest levels in light. This indicates that there may be a constant level of lipid peroxidation even in the dark and continuous leakage of Ca^{2+} and other ions into the tissues accompanied by a low level of Ca^{2+}ATPase pump activity. In light the activity of the Ca^{2+} pump increases and this may explain the higher calcification rate in light for ahermatypic corals. Zooxanthellate corals would experience even higher rates of calcification during the day as a result H_2O_2 build-up, increased lipid-peroxidation and leakage of more Ca^{2+} into the calicoblastic layer. The results and elements of the above discussion are combined into a model, following Adkins et al (2003), presented in Figure. 7.

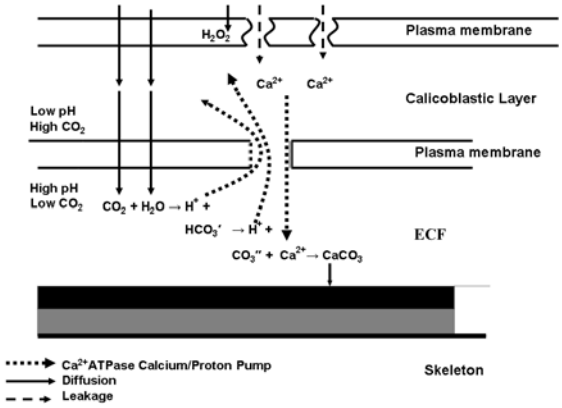

Figure 7: A calcification model for diurnal banding. Low levels of intracellular Ca^{2+} are maintained by the Ca^{2+}ATPase/proton pump which is light sensitive and maintains a lower level of Ca^{2+} in light. H_2O_2 produced by zooxanthellae builds up during the day, causes lipid peroxidation of the plasma membrane, makes it leaky to Ca^{2+} and provides a route for more Ca^{2+} (and other ions) to enter the calicoblastic layer. Secretion by the calicoblastic layers of a new layer of organic matrix takes place during the night. It has Ca^{2+} binding and enzymatic properties which enhance adhesion and the formation and deposition of $CaCO_3$. Ca^{2+} is actively transported into the ECF by the Ca^{2+}ATPase pump and protons are transported in the opposite direction. The removal of protons enhances the formation and deposition of $CaCO_3$ and the pH changes enhance the diffusion of CO_2 into the ECF. There is greater deposition on the new layer of the organic matrix during the day. This results in alternating low and high density laminations.

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Mineral phase of COCs and fibers in coral skeletons

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Abstract. The variation in the Sr/Ca ratio of coral skeletons is sensitive to changes in temperature and presents an archive for reconstructing the paleo-seawater environment. Information on the mineral phases corresponding to coral skeletal textures from micro- to nano-meter scales would be helpful toward resolving the mechanism causing the variation of Sr/Ca ratio. To confirm mineral phase differences in coral skeletons, we conducted the mineral phase identification in microstructures and nano-textures of the coral *Porites lobata* by X-ray diffraction analysis with synchrotron radiation (SR-XRD) and transmission electron microscope (TEM), respectively. Coral skeletal microstructures consist of centers of calcification (COC) and fibers. COCs are < 30 μm in diameter in the samples. The X-ray beam size of SR-XRD collimated of 15 μm and 40 μm in diameter enabled us to obtain the information of mineral phase in COCs and fibers separately. All diffraction peaks of COCs and fibers can be explained as aragonite with the lower detection limit of ca. 0.5 wt% of strontianite by SR-XRD. All TEM observations also indicate the aragonite phase in coral skeletal nano-textures.

Key words: Mineral phase, SR-XRD, Coral, Skeleton, Microstructures

Introduction

The strontium/calcium (Sr/Ca) ratio in coral skeletons varies with surrounding temperature change has and thus may provide an accurate paleo-thermometer of sea surface temperature (SST) (de Villiers et al., 1995). Coral skeletons are reportedly composed of only aragonite so that the Sr heterogeneity derives from the compositional rates of solid solution of aragonite.

Greegor et al. (1997) reported that as much as 40% of strontium (Sr) in coral aragonite existed as strontianite ($SrCO_3$) by using X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). The presence of strontianite in coral skeletons could significantly influence Sr/Ca paleo-thermometry because the thermometry assumes the strontium in coral aragonite exists in solid solution. Greegor et al. (1997) implied that Sr did not substitute simply for Ca, and therefore coral Sr/Ca thermometry could not be applied for paleo-environmental analysis. Indeed, coral skeletons contain higher amount of Sr than expected by the solubility limits calculated from synthetic aragonite (Plummer and Busenberg, 1987). On the other hand, EXAFS analyses performed by Finch et al. (2003a, 2003b) and Allison et al. (2005) showed no evidence of the presence of strontianite or its intermediate state in coral aragonite. However, neither XANES nor EXAFS can identify the mineral phase directly.

Recent advances of micro analytical methods allow the analysis of trace elements within the coral skeletal micrometer scale structures (microstructures) that correspond to daily growth resolution (Meibom et al. 2008). Cohen et al. (2001) focused on the substantial heterogeneity of Sr/Ca components in coral skeletal microstructures, which were not solely temperature related. Previous studies explained this heterogeneity as resulting from biological effects, kinetic effects, and different mineral phases. However, no model has yet been generated to explain the effects of biological, kinetics and mineral phases on the observed chemical and isotopic variations. Identification of the mineral of the coral skeletal microstructure and nano-meter scale structures (nano-textures) are thus crucial for explaining these effects.

Few studies on the mineral phase in coral skeletal microstructures have been performed (Vongsavat et al. 2006, Stolarski et al. 2007, Przenislo et al. 2008). Stolarski et al. (2007) were the first to report the mineral phase of coral skeletons, extracted as *en bloc* without pulverization, by high-resolution synchrotron radiation diffraction. However, their study did not analyze the mineral phase corresponding to coral skeletal microstructures.

Here we report the mineral phase identification in coral skeletal microstructures and nano-textures using X-ray diffraction analysis with synchrotron radiation (SR-XRD) and transmission electron microscope (TEM). Microstructures of coral skeletons consist of two morphology; COC (centers of calcification) and fibers (fasciculi) (Partz, 1882; Ogilvie, 1886). The SR-XRD analysis method enables one to identify the

mineral phase of COC and fibers by X-ray spots, 15 μm and 40 μm respectively. Our study provides the first analysis of the mineral phase in coral skeletal microstructures and nano-textures.

Material and Methods

All data were obtained from the scleractinian coral *Porites lobata* collected from Ishigaki Island, Okinawa prefecture, Japan in 2005. The coral samples were prepared by first removing the tissue layers with water and allowing to dry at ambient temperature. Each sample was then cut, rinsed with distilled water and dried at room temperature. Samples were embedded in epoxy resin (Logitech Ltd.) and polished.

Microstructures in the coral skeleton were examined using scanning electron microscopy (SEM) in a section cut vertical to the axis of growth direction on a HITACHI S-3000H at Hokkaido University. Acceleration voltages of the electron beam were 10kV. All samples were polished and etched with 0.5MHCl (5-second), then sputter-coated with a thin layer of platinum.

We performed SR-XRD analysis to identify the mineral phase in coral skeletal microstructures. Before the SR-XRD analysis, we ensured the analysis area was free of micro-holes, then bonded the epoxy resined coral skeletons to the glass slide and polished them < 100 μm thickness. In previous studies, the samples could not be separated from slide because of the thinness of the sample. All the samples have been prepared under the room temperature and were peeled from the glass slides. The experiments were conducted with an imaging plate (IP) on BL-18c at Photon factory, KEK (high energy accelerator research organization), Tsukuba, Japan. A monochromatic incident X-ray beam with a wavelength of 0.616Å was used and collimated to a diameter of 15 μm or 40 μm. These analytical spots were precisely positioned by CCD camera, allowing the spatial distribution in coral microstructures to be directly investigated. The two-dimensional IP data were integrated and conducted to one dimensional intensity data.

Nano-textural observations and diffraction patterns of coral skeletons were performed by Transmission electron microscope (TEM) on JEOL JEM-2010 operated at 200kV. An ultra-thin section of the sample was prepared by finely polishing and Ar ion sputtering. No calcium oxide (CaO) in this analysis indicated that the original mineral phase had been maintained in the coral sample.

In order to verify the detection limit of strontianite in coral aragonite by using SR-XRD, we prepared the synthetic aragonite and strontianite minerals (Kanto chemical co., Inc).

The Sr-free aragonite powder was prepared by precipitation from aqueous solutions (modified from Lucas-Girot *et al*., 2007). A solution of potassium hydrogen carbonate, 0.1 M, was prepared through the dissolution of K_2CO_3 (Wako Pure Chemical Industries, Ltd.) in distilled water. A calcium chloride solution, 0.1M, was prepared by dissolving $CaCl_2 \cdot 2H_2O$ (Kosa Chemical Co., Ltd.) in distilled water. The boiling solution of calcium chloride was added to the stirred boiling solution of potassium hydrogen carbonate. The precipitate was aged 5 min in the boiling mother solution and then filtered, washed with boiling water, and dried 110℃ over night. An XRD pattern showed that powder included aragonite and a little calcite.

We repeated the above cycle, but in the second cycle we added a small amount of aragonite powder, obtained in the first cycle, as seed crystals to the mother solution. The XRD pattern of this powder produced only aragonite peaks.

After the aragonite was synthesized, we intermixed the strontianite to the synthetic aragonite with 0, 0.117, 0.49, 1.26, 3.53, 5.25, 10.10 wt% respectively (see Table.1).

Results and Discussions

Implication for Sr/Ca thermometry

Fig. 1 shows coral microstructures of *Porites lobata* observed by SEM and optical microscope. In this study, the sizes of the COC are about 5-30 μm in diameter. Fig. 2 indicates one-dimensional intensity

Sr (wt %)	$SrCO_3$ (wt %)	$CaCO_3$ (mg)	$SrCO_3$ (mg)
5.99	10.1	20.07 ± 0.01	2.25 ± 0.01
3.12	5.25	23.42 ± 0.01	1.30 ± 0.01
2.10	3.53	104.29 ± 0.01	3.82 ± 0.01
0.75	1.26	105.38 ± 0.01	1.35 ± 0.01
0.29	0.487	106.26 ± 0.01	0.52 ± 0.01
0.07	0.117	109.18 ± 0.01	0.128 ± 0.01

Table.1: The amount of strontianite in the synthetic aragonite.

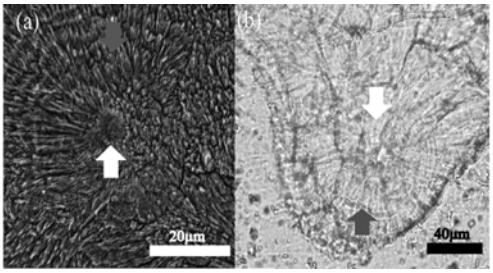

Figure.1: Coral skeletal microstructures of *Porites lobata* in scanning electron microscope (SEM) image (a) and petrographic thin section (b). The white arrow indicates centers of calcification (COC) and the gray arrow shows the emergent bundles of aragonite fibers surrounding the COC. Modified from Sowa *et al*. 2008.

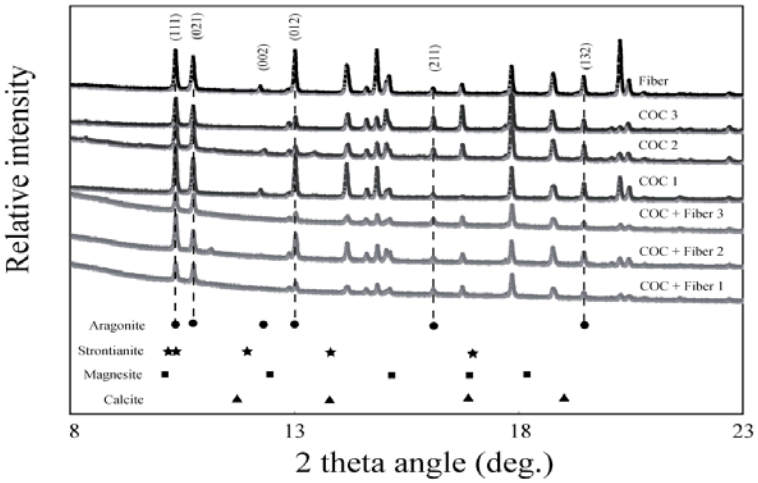

Figure.2: X-ray diffraction patterns of coral skeletal microstructures. Three of diffraction traces were analyzed by 40 μm spot size in diameter (COC + Fiber1-3) and the others by 15 μm (COC 1-3, Fiber). Aragonite, strontianite, magnesite and calcite are represented by solid circle (●), star (★), square (■), and triangle (▲), respectively.

patterns from 8 to 23 degrees 2-theta of the mineral phases in coral skeletal microstructures. The X-ray laser spot sizes of this study are 15 μm and 40 μm respectively, which correspond to the diffraction patterns of COC at three points, fiber of a single point, and an area that includes COC and fibers of three points. These diffraction patterns illustrate that COC and fibers consist only of aragonite.

To examine the mineral phase in coral skeletal nano-textures, we conducted TEM observations in COC and fibers. Coral skeletal nano-textures contain submicron, granular or needle-like shaped grains as shown in Fig. 3. The grain size of coral aragonite by our TEM observation resemble the nano-scale grain size observed by AFM (Cuif and Dauphine 2005). In the coral skeletal nano-textures, we did not find evidence for strontianite in the diffraction patterns of the coral skeletons (Fig. 3).

Fig. 4 illustrates the X-ray diffraction patterns of the six mixture samples of strontianite ($SrCO_3$) and the synthetic aragonite ($CaCO_3$) by SR-XRD. To detect the strontianite by qualitative analysis, we calculated and plotted the relative intensity of strontianite (111) normalized by aragonite (111). The regression line is $Y = 2.83*X$ ($R^2 = 0.96$), which was calculated from samples except 0.117 wt%. The relative intensity of 0.117 wt% was - 0.0673, which was under that of 0 wt%. This experimental result indicates that the lower limit of detection of strontianite in the synthetic aragonite is approximately 0.5 wt%, which corresponds to a Sr content of 2900 ppm (see Table 1). Greegor et al. (1997) reported that 3000 ppm (40 % of 7500 ppm) of Sr presented as strontianite. SR-XRD could detect the strontianite in coral skeletal microstructures if coral skeleton contains 3000 ppm of strontianite.

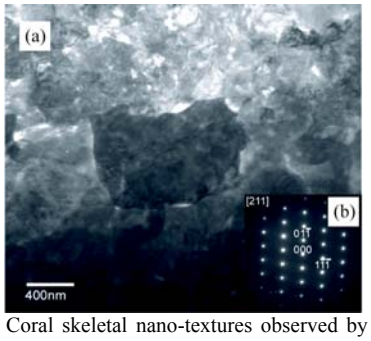

Figure.3: (a) Coral skeletal nano-textures observed by TEM. (b) Electron diffraction pattern of coral aragonite in a selected area.

In our study, however, with lower detection limits of approximately 0.5 wt% of strontianite and without any impurity phase conditions (Fig. 2), diffraction patterns in coral skeletal microstructures present only Bragg peaks at positions corresponding to the aragonite diffraction patterns. We could not detect strontianite and other mineral phase in the coral nano-textures from the diffraction patterns (Fig. 3). Our mineral phase analysis of coral skeletal microstructures and nano-textures are similar to the results of EXAFS and XANES, which showed no existence of strontianite or it's intermediate state in coral skeletons (Finch *et al*., 2003a, 2003b; Allison *et al*., 2005).

Cohen et al. (2001) and Allison et al. (2005) reported that COC contain higher Sr/Ca ratio than fiber. However, there is no significant difference in the mineral phases of COCs and fibers in this study. These results imply that the Sr heterogeneity between COCs and fibers is not due to a difference in mineral phases. The causes of Sr heterogeneity are unclear and the mechanism of biological or kinetic effects remains to be resolved.

In summary, all diffraction patterns of coral skeletal

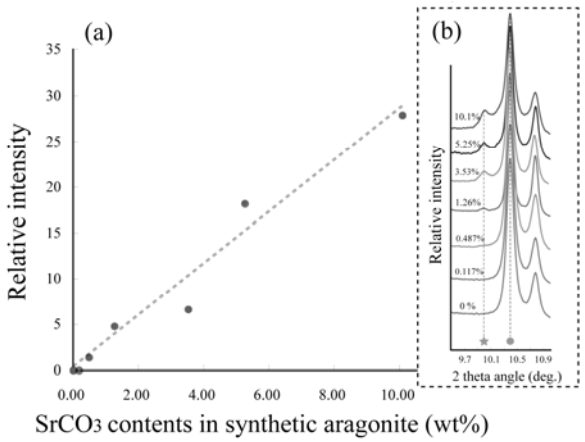

Figure. 4: (a) Experimental detection limit of strontianite ($SrCO_3$) by SR-XRD. Table.1 shows each contents of strontianite. Y-axis indicates that the relative intensity of strontianite (111) normalized by that of the synthetic aragonite (111). (b) The X-ray diffraction patterns of contents of strontianite against the synthetic aragonite; 0, 0.117, 0.49, 1.26, 3.53, 5.25 and 10.10 wt% respectively (see Table.1). Aragonite (111) and strontianite (111) indicate solid circle (●) and star (★) respectively.

microstructures and nano-textures (Figs. 3, 4) indicate that there is no evidence of strontianite in coral skeletons (within the lower limits of detection discussed above) and strontium is likely incorporated into the aragonite as solid solution.

The presence of strontianite remains possible, because our analysis focused only on limited areas in coral microstructures and nano-textures and we did not examine other coral species. Greegor et al. (1997) noted the presence of strontianite in *Montastrea annularis* and *Acropora palmata*. Here we investigated *Porites lobata* because it has been used as a paleo-environmental archive. It is possible that *Montastrea annularis* and *Acropora palmata* contain strontianite in their skeletons. Shirai et al. (2008) reported that Sr is heterogeneous in *Acropora nobillis* skeletal microstructures. Further investigation of the mineral phases of *Acropora* using SR-XRD may be useful.

Mineral phase analysis by SR-XRD and TEM corresponding to the coral skeletal microstructures and nano-textures show solely the aragonite diffraction pattern in this study. These results indicate that Sr/Ca thermometry of coral *Porites* should not consider the presence of strontianite in coral skeletons.

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The authors acknowledge Nomura H, Nakamura K at thin section technician's lab, Hokkaido University for technical advising of sample preparation, and Dr. Hagino K and Omori K for supporting this experiment. We also thank Dr. Joan Kleypas and two anonymous reviewers for comments, which significantly improved this manuscript. The synchrotron X-ray diffraction studies at BL-18C in PF were performed under the approval of the Photon Factory Advisory Committee (proposal no.2007G042).

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Applications of the SEDCON and FORAM Indices on patch reefs in Biscayne National Park, FL, USA

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Abstract. Coral cover remains highest on patch reefs at the northern end of the Florida reef tract. Two indices, the FORAM Index and the SEDCON Index, were developed to indicate the suitability of a reef environment for continued reef accretion. Patch reefs were sampled in Biscayne National Park, FL, to assess sediment characteristics and foraminiferal assemblages, as well as to examine trends between the two indices. Sediments associated with a majority (59%) of reefs were coarse sands; muddy sediments were restricted to a few inner patch reefs that were isolated from the flow of Caesar's Creek. Unidentifiable grains dominated the sediment constituents, along with calcareous algae and molluscan debris. Shells from 82 genera of Foraminifera were identified in the sediments. *Quinqueloculina* was the most consistently common genus. Percent mud was the most influential measured variable on the distribution of both sediment constituents and foraminiferal assemblages. Patterns of FORAM and SEDCON Index values and their similarity to temperature, salinity, and percent mud distributions show that Caesar's Creek is affecting the benthic community by providing flow that limits the accumulation of mud and potentially other anthropogenic stressors. Overall this study suggests that the reefs in this area are marginal for continued reef growth.

Key words: Foraminifera, SEDCON, FORAM, reefs, sediments.

Introduction

The Florida Fish and Wildlife Research Institute's Coral Reef Evaluation and Monitoring Program (CREMP) has monitored the decline of coral in Florida for over ten years. Their study has shown that coral cover remains highest on patch reefs at the northern end of the reef tract (16% coral cover) (Beaver et al. 2005). The reasons for this trend are not well understood, but may be related to the protection from extreme variations in water quality parameters provided by the near constant presence of islands at the north end of the Florida Keys.

Patch reefs were sampled in Biscayne National Park, FL (Fig. 1), to assess environmental patterns and test two environmental indices. Reefs in this area are protected from direct influence of Biscayne Bay by the presence of Elliot Key and Old Rhode's Key. However, Caesar's Creek, between the two Keys, has a known net outflow of water from the Bay directly onto the reefs.

Two indices have been developed based on Foraminifera and sediment constituents. The FORAM Index and the SEDCON Index were developed to relate the response of the calcifying benthic community to the status and suitability of the environment for future reef growth (Hallock et al. 2003, Daniels 2005). In both indices, shifts in assemblage contribution from mixotrophic organisms (symbiont-bearing foraminifers and coral) to heterotrophic organisms (smaller foraminifers and molluscan fragments) are hypothesized to reflect environmental changes associated with reduced potential for coral reefs to dominate and recover from stress events. The low-cost, easily applied nature of these indices provides a potential new tool for resource managers, especially in regions where technically trained personnel are more affordable than chemical analyses.

Figure 1. Sampling location within Biscayne National Park, FL

Material and Methods

Sediment samples and environmental data were collected from 32 reefs in Biscayne National Park, including 30 patch reefs and two bank-barrier reefs (Pacific Reef and Lugano Reef).

Sediments were wet sieved and the sand fraction was dried. A one gram subsample was removed for the FORAM index analysis. Grain-size analysis was performed on the remaining sample fraction. The 0.5-1mm and 1-2mm size fractions were recombined and a one gram subsample was taken for analysis for the SEDCON index.

For the FORAM Index, up to 150 foraminifers were picked from the 1g subsample. Each specimen was then identified to genus. For the SEDCON Index, 300 grains were randomly chosen from the subsample using a point-count method. Each grain was then identified to the extent possible.

The gathered assemblage data were then used to calculate FORAM and SEDCON Index values according to Tables 1a and 1b. An FI value >4 indicates water quality suitable for dominance by calcifying organisms with algal symbionts. A value from 2-4 indicates conditions where calcifying symbioses can persist but not dominate and may not be able to recover from a stress event. At <2, water quality is probably not suitable for reef growth (Hallock et al. 2003). SI values reflect sediment contributions: SI values >4 reflect sediment production dominated by calcifying symbioses (i.e., coral and foraminifers). From 2-4, dominance is by autotrophic and heterotrophic processes (calcareous algae and non-symbiotic animals); SI <2 reflects dominance by unidentifiable grains presumably reflecting bioerosion.

The SIMPROF and SIMPER tests in PRIMER statistical software (Clarke and Warwick 2001) were used to identify similarities between samples based on assemblage composition for both the foraminiferal assemblages and the sediment constituent assemblages. Surfer surface mapping system and ArcMap were then used to plot the similarity data as well as to create contour lines for temperature, salinity, percent mud, FORAM Index and SEDCON Index values.

Results

Spatial patterns were determined by calculating Moran's I value of spatial autocorrelation (Anselin et al. 2006). A significant value indicates non-randomness. All three environmental variables had significant spatial autocorrelation ($p<0.05$) (Mud - 0.42, Temperature - 0.31 and Salinity - 0.27 respectively). Temperature and salinity negatively correlated with each other (bivariate Moran's I: -0.33). Percent mud also had significant correlations with temperature and salinity (Pearson's ρ = -0.42, 0.53 respectively). The three environmental parameters also spatially correlated with the FI and SI values with significant Pearson's correlations ($p<0.05$) (Figs. 2, 3, and 4).

Table 1. Calculations for a) the FORAM Index and b) the SEDCON Index.

a)

$FI = (10*P_s)+(P_o)+(2*P_h)$	
Where,	$P_s = N_s/T$ $P_o = N_o/T$ $P_h = N_h/T$
And,	T = total number of specimens counted
	N_s= number of symbiont-bearing Foraminifera
	N_o=number of stress-tolerant Foraminifera
	N_h= number of other small, heterotrophic Foraminifera

b)

$SI = (10*P_c)+(8*P_f)+(2*P_{ah})+(0.1*P_u)$	
Where,	$P_c = N_c/T$ $P_f = N_f/T$ $P_{ah} = N_{ah}/T$ $P_u = N_u/T$
And,	T = total number of grains counted (300)
	N_c= number of coral grains
	N_f=number of symbiont-bearing Foraminifera
	N_{ah}=number of coralline algae, calcareous algae, and heterotrophic skeletal grains
	N_u= number of unidentifiable grains

Foraminiferal Analysis

Statistical tests in PRIMER showed sites clustered into five major groups (A, B, C, D, E) based on the foraminiferal assemblage (Fig. 5). Table 2 shows the averages for the environmental data within each cluster as well as the percent contribution by certain foraminiferal groups.

FORAM Index values were calculated in accordance to the formula presented in Table 1a for only those sites where more than 50 specimens were present in a one gram sample. The highest FI values (Fig. 5), correspond to SIMPER groups A and E, with Pacific Reef having the highest average FI value (7.0, SD=1.9). Lowest FI values correspond to SIMPER group C. The lowest FI value was at Bug Reef with an FI of 2.1 (SD=0.12).

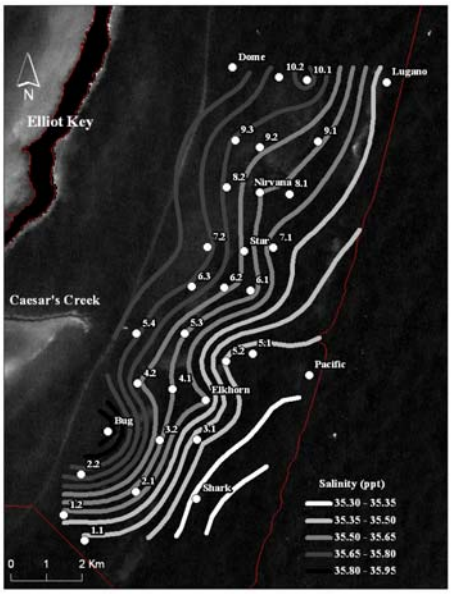

Figure 2. Contour lines of salinity values in the sampling area in Biscayne National Park. The less than 1ppt change over the sampling area indicates that this is not a major factor in shaping the indices patterns.

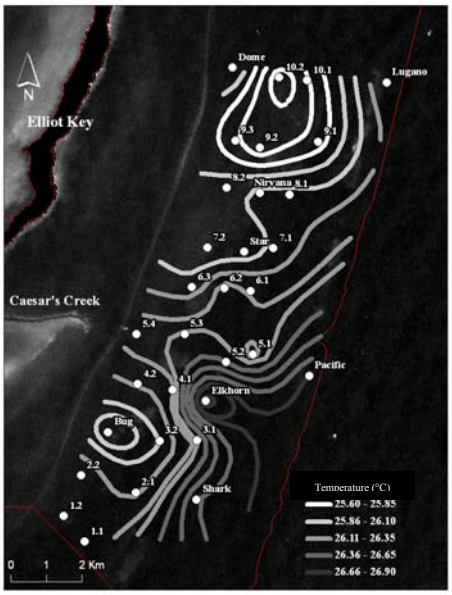

Figure 3. Contour lines of temperature values throughout the sampling area in Biscayne National Park. The less than 2°C change over the sampling area indicates that this is not a major factor in shaping the indices patterns.

Sediment Constituent Analysis

Only two clusters were identified in the sediment constituent data. Both groups were dominated by calcareous algae, mollusks, and unidentifiable grains. Unidentifiable grains dominated Group B which were located in higher energy areas. Table 3 summarizes the environmental data for each sediment constituent defined cluster.

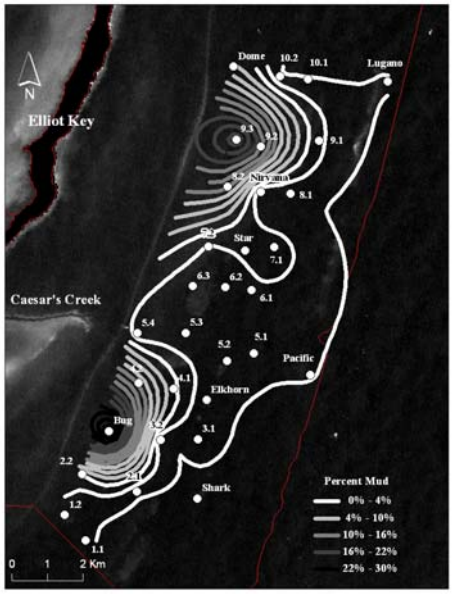

Figure 4. Contour lines of percent mud values throughout the sampling area in Biscayne National Park. The absence of mud offshore of Caesar's Creek and abundance of mud offshore of the Key islands may be influencing, or may indicate another influence that is shaping the patterns seen in the indices.

Unidentifiable grains had a strong negative Pearson's correlation (-0.72) with percent mud, while at the muddiest sites there were more identifiable calcareous algae, which positively correlated to the SI values (0.67)

SEDCON Index values were calculated based on the equation derived in Daniels (2005) and shown in Table 1b. The range of mean values among the reefs was from 0.64 (SD=0.03) at Shark Reef to 2.48 (SD=0.42) at Reef 9.3. The lower SI values are found in the vicinity of Caesar's Creek, while the reefs furthest from direct sources of water flow have higher SI values (Fig. 6).

Discussion

Waters emerging from Biscayne Bay through Caesar's Creek into the study area clearly influence temperature, salinity, sediment texture, and the foraminiferal and sediment -constituent assemblages as reflected in the FORAM and SEDCON Indices.

The SEDCON Index was faster and easier to apply, while the FORAM Index produced more inter-reef detail. However, there was an unanticipated negative Pearson's correlation between the FORAM Index and the SEDCON Index (-0.53). Spatially, the patterns between the two indices co-varied significantly with a Moran's I value of -0.399 (p=0.01).

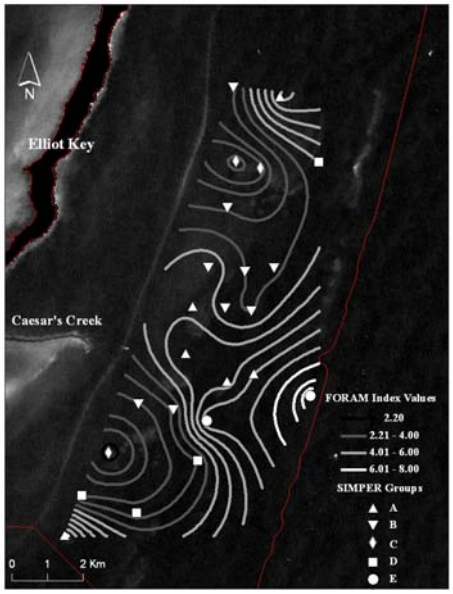

Figure 5. Reefs that clustered together based on similarity of foraminiferal assemblage are indicated with the same symbol. Five cluster groups were identified. Contour lines represent FORAM Index values throughout the sampled area of Biscayne National Park.

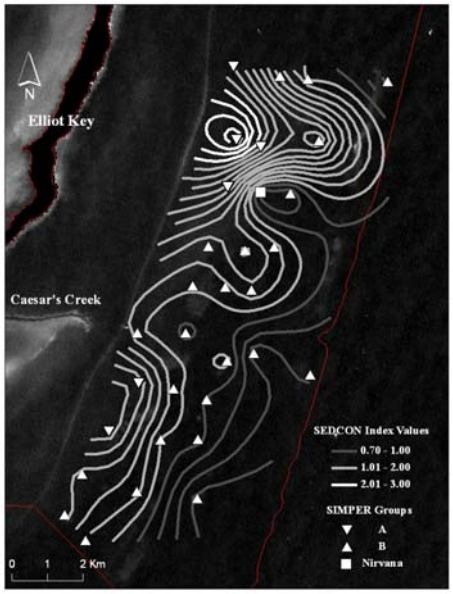

Figure 6. Reefs that clustered together based on similarity of sediment constituent assemblage are indicated with the same symbol. Two cluster groups were identified. Contour lines represent SEDCON Index values throughout the sampled area of Biscayne National Park.

The negative correlation between the two indices appears to reflect the difference between the potential for carbonate production by symbiont-bearing corals and foraminifers, as indicated by the FORAM Index, versus the reality of the decline of coral cover over the past several decades (e.g., Dupont et al. in press), which is reflected in the SEDCON Index. Water quality in the reef area of Biscayne National Park appears to be suitable for calcifying symbioses; the average FI value across all reefs was 4.12. However, the average SI value of 1.26 indicates that potential is not being realized and that erosional processes, and secondarily autotrophic and heterotrophic production, are dominant sources of carbonate sediments associated with BNP patch reefs.

The divergence between the two indices likely indicates that climatic (e.g., hurricanes), regional (e.g., coral diseases) and global (e.g., photo-oxidative stresses) influences are more important than local water quality in the decline of Biscayne patch reefs. Populations of the shorter-lived foraminifers can rebound from mortality events in a few years (Hallock et al. 2006), while long-lived corals have not been able to rebound from successive impacts and mortality events over the past several decades (e.g., Dupont et al. in press).

This study expands upon the original premise of the SEDCON Index, when used in conjunction with the FORAM Index. They were originally both thought to indicate whether water quality supports calcifying symbioses. But this study indicates that the FORAM Index does so, while the SEDCON Index indicates that factors other than water quality can control sediment composition, reflecting more of the history of processes affecting macrobenthos.

Table 2. Summary table of the environmental data averaged for each of the five clusters based on similarity of foraminiferal assemblages, and the % contribution of certain foraminiferal groups to each cluster.

Group	FI	Density (shells/g)	# of Genera	pH	Temperature	DO	Salinity	% Mud	Phi	% Symbiont-bearing	% Stress-tolerant	% Other taxa
A	4.85	126	21.7	8.31	26.09	6.32	35.57	0.55	1.11	34.76	5.24	51.2
B	3.60	957	31.7	8.32	26.08	6.25	35.66	5.76	1.33	22.71	9.12	59
C	2.22	5518	29.8	8.22	25.83	6.42	35.82	26.4	2.75	5.16	21.7	63.5
D	3.13	1015	33.0	8.26	26.11	6.46	35.60	1.95	0.75	15.9	11.7	63.37
E	6.36	123	25.3	8.29	26.81	6.79	35.44	0.30	0.67	54.8	0	36.23

Table 3. Summary table of the environmental data averaged for the two clusters based on sediment constituent assemblages.

Group	SI	pH	Temperature	DO	Salinity	% Mud	Phi
A	1.89	8.26	25.95	6.29	35.75	17.3	2.00
B	1.13	8.29	26.20	6.38	35.58	1.10	0.88
Nirvana	0.85	8.25	26.09	6.43	35.60	0.06	1.00

Conclusions

1. The patterns of salinity, temperature, and percent mud reflect waters emerging from Biscayne Bay through Caesar's Creek into the study area.
2. The influence of the water emerging from the bay is reflected in the FORAM and SEDCON Indices.
3. The SEDCON Index was faster and easier to apply, while the FORAM Index produced more inter-reef detail.
4. The negative correlation between the two indices may reflect the difference between the potential for carbonate production by symbiont-bearing corals and foraminifers, as indicated by the FORAM Index, versus the reality of the decline of coral cover over the past several decades, which is reflected in the SEDCON Index.
5. Water quality in the reef area of Biscayne National Park appears to be suitable for calcifying symbioses; the average FI value across all reefs was 4.12.
6. The average SI value of 1.26 indicates that erosional processes, and secondarily autotrophic and heterotrophic production, are dominant sources of carbonate sediments associated with BNP patch reefs.
7. The divergence between the two indices may indicate that regional (e.g., coral diseases) and global (e.g., photo-oxidative stresses) influences are more important than local water quality in the decline of Biscayne patch reefs.

Acknowledgement

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Session number 4

The significance of geochemical proxies in corals, does size (age) matter?

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Abstract. The main goal of this paper is to determine how size (small vs. large coral heads) and age (juvenile vs. adult) potentially affect geochemical proxies in coral skeleton, widely used in paleoclimate studies. After ensuring that corals are not diagenetically altered, we analyse Sr/Ca, Mg/Ca, $\delta^{18}O$ and $\delta^{13}C$ on 3 cores from two study areas. We compare two different size corals from similar environmental setting in Vanuatu. The smaller coral head, despite presenting a similar seasonal shape pattern that its larger counterpart, presents higher Sr/Ca, potentially interpreted as cooler temperatures. We also study the top and the basement of a ~7m-high massive *Porites* sp. core from New Caledonia that reveal both modern and post-settlement periods. A sharp evolution of the geochemical proxies is seen during the post-settlement period. The differences in between the cores seem unlikely to be caused by environmental factors alone and we argue for biological differences. The implications are important for fossil studies where coral cores are often recovered in small pieces with no age indices (adult or young). Geochemical reconstructions should then be carefully interpreted as ontogenic effects could easily bias the results.

Key words: coral, geochemical proxies, paleoclimate reconstruction

Introduction

Massive coral have received growing attention in paleoclimate studies over the years. Coral carbonate skeletons are useful archives containing multiple proxies that could help reconstructing environmental history. In most coral studies, a calibration phase linking geochemical signals to environmental parameters is achieved on the modern part of a core. Calibration equations are then applied to the whole core (Ourbak 2006) or to a fossil piece (Corrège et al. 2004) to evaluate paleoclimate variability. Calibration studies often invokes thermodynamic laws predicting a temperature-related incorporation of trace elements (Sr/Ca for example) and oxygen isotopes ($\delta^{18}O$) in calcium carbonate (Kinsman and Holland 1969; Weber and Woodhead 1972). Calcification in bioconstructed minerals such as coral aragonite have complex incorporation processes and a "vital" effect is invoked to account for biological effects perturbing the pure thermodynamic relationship (Erez 1978).

Several studies have been conducted on biological effects, but only a few indirectly address the potential impact of coral size or age (different growth stages) on the geochemical signals. However, geochemical composition of several marine carbonate species such as benthic and planktonic foraminifera (Bijma et al. 1998; Hintz et al. 2006), or bivalves (Gillikin et al. 2007) present ontogenic effect. Concerning corals, a potential size-effect on the accuracy of Sr/Ca=f (SST) calibration has been published (Marshall and McCulloch 2002); SST stands for sea surface temperature. A comparison of several coral heads along the Great Barrier Reef produced different calibrations. Marshall and McCulloch (2002) suggested that younger corals are less reliable than larger/older corals because of some physiological difference.

Fossil corals are often recovered out of growth position or as fragments. Thus, it is important to study the potential effect of size/age on skeleton geochemical composition as a potential bias to environmental reconstructions. After ensuring that the cores present pristine aragonite, we interpret geochemical results (Sr/Ca, Mg/Ca, $\delta^{18}O$, $\delta^{13}C$).

First, we address the importance of the size of coral heads. We selected two Vanuatu coral heads of different sizes and compare their Sr/Ca composition. Small coral heads capture temperature signal as well as their larger counterparts but the two series present a systematic difference.

Second, we test the age-effect by determining how reliable are the geochemical signals at the basement of a coral core, corresponding to the post-settlement period. We use a ~7m-high massive colony from New Caledonia. At the bottom of the core, 23-years of distinct banding patterns give crucial information on coral post-settlement period.

Material and Methods

Material

All the cores were recovered by SCUBA, sliced, cleaned in deionized water ultrasonic bath and then air-dried.

Two *Porites lutea* cores from Espiritu Santo, Republic of Vanuatu were sampled. A large (~140 cm long) coral head was cored in 1992 within Malo Channel (15.7ºS, 167.2ºE) and described by Kilbourne et al. (2004a; 2004b). The second colony (22 cm high) was collected by hand in July 1979 offshore of Tasmaloum, 34 km away. Chronology established by band counting on X-rays give ages of 65 years and 11 years, respectively, based on a 2cm/year growth rate for both cores. Even though the two sites are situated in similar oceanic settings, episodic terrestrial inputs during the rainy season (May to September) could be important at Tasmaloum site (F. Taylor, pers communication). These episodes affect salinity signal but are usually hours to daylong and should not affect our monthly sampling resolution.

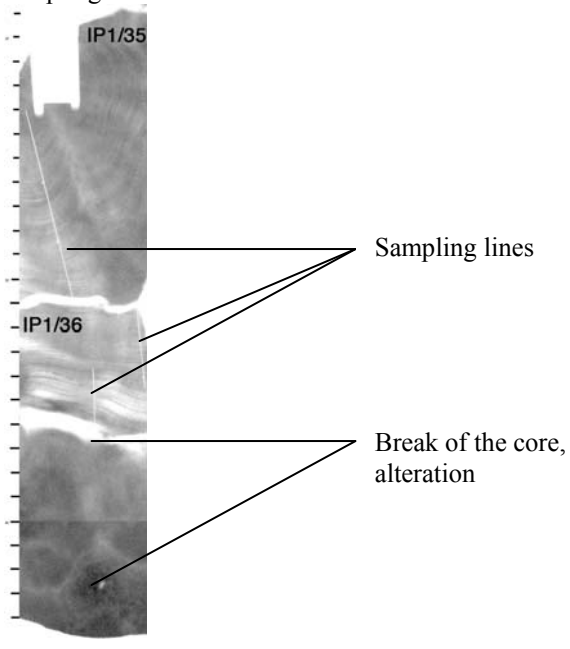

Figure 1: X-Ray of IP1/35 and IP1/36 slices at the very bottom of the New Caledonia coral. Scale on the left: 1 cm in between two ticks. The last 8 cm have been discarded due to absence of clear growth bands and presence of alteration features at the very bottom of the core.

A ~7m-high *Porites sp*. core was collected in 2003 on the outer reef of Ile des Pins, New Caledonia (22.31ºS, 167.25ºE). Results of the more recent 567 cm are discussed in Ourbak (2006). Deeper than 567 cm, about a meter of the core was discarded due to erratic growth patterns. This study focuses on the last two bottom slices (Fig. 1), undated, at the basement of the core, which we expect to correspond to the post-settlement period and the initial growth period of the colony. We chose a constant 0.5 cm per sample (corresponding to a pseudo-half year resolution). A narrow zone of preferential dissolution and complex growth band patterns at the bottom position of the core led us to stop sampling 8 cm before the bottom of the core (slice named IP1/36, Fig. 1).

Methods

Vanuatu Sr/Ca measurements were made using a PerkinElmer Optima 4300 DV ICP-OES (CMS, University of South Florida), following the method for drift corrected from Schrag (1999). An Optima mass spectrometer was used to produce New Caledonia $\delta^{18}O$ and $\delta^{13}C$ data (UMR EPOC, Université Bordeaux 1). New Caledonia Sr/Ca and Mg/Ca ratio were determined with a PerkinElmer Optima 3000 DV ICP-AES (IRD Nouméa). A Siemens D500 diffractometer and a Cambridge Stereoscan 200 scanning electron microscope were used for XRD analysis and SEM images, respectively, at IRD Bondy Ile de France center.

Results and Discussion

Potential diagenetic alteration

Diagenetic features in corals such as secondary deposition of aragonite needles and dissolution processes alter Sr/Ca, Mg/Ca, $\delta^{18}O$ and $\delta^{13}C$ values in fossil cores and even in the very recent part of modern cores (Bar-Matthews et al. 1993; Hendy et al. 2007). This could bias paleothermometers by cooling the temperature as much as 5 ºC (Muller et al. 2001).

XRD (not shown here) does not detect calcite and confirms that New Caledonia coral is made of aragonite. Moreover, SEM image (Fig. 2) presents unaltered primary aragonitic needles with no indications of dissolution. In addition, level of correlation between geochemical tracers increase for altered crystals compared to pristine ones (see Quinn and Taylor (2006), for example). We computed correlation coefficients and did not find any increase of the correlation over the bottom samples (Table 1), ruling out any diagenesis impact on New Caledonia coral. Similarly, The Malo channel Vanuatu core has been previously checked (SEM and petrographic microscope) and do not present diagenetic alteration (Kilbourne et al. 2004a).

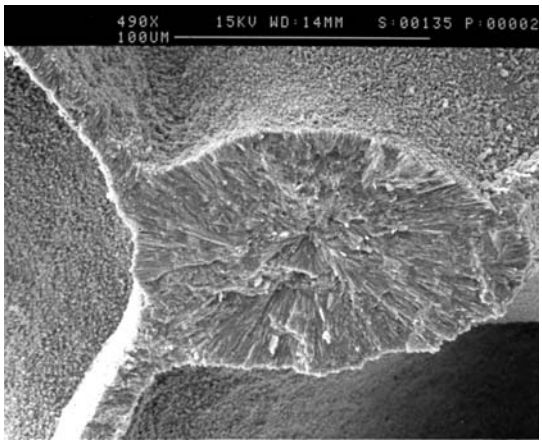

Figure 2: IP1/35 SEM image presents well-preserved dissepiments, broken septa and aragonite needles arguing for a pristine skeletal material, without alteration.

Sr/Ca	*-0.45*	*0.47*	*-0.42*	*-0.60*
-0.04	**Mg/Ca**	*-0.49*	*-0.28*	*-0.14*
0.40	0.07	**δ^{18}O**	*-0.09*	*-0.74*
-0.69	0.07	-0.17	**δ^{13}C**	*0.74*
-0.72	-0.001	-0.77	0.76	**δ^{13}Ct**

Table 1: Correlation coefficients (*r*) between the different geochemical proxies measured in New Caledonia core (*$r > 0.38$, $p < 0.5$, $n = 25$*). δ^{13}Ct stands for $\delta^{13}C_{transformed}$, see text for details. Top (modern) samples are on the right side, in italic while bottom (older) are on the left.

Does size matter? The Vanuatu corals

Marshall and McCulloch (2002) found large differences in Sr/Ca=f (SST) calibration equations from the Great Barrier Reef modern coral heads. They suggest that smaller corals produce less reliable calibrations than larger ones and point that "immature or juvenile coral heads are somehow physiologically different from their more mature counterparts, and this is reflected in their intake of trace elements and isotopes." To our knowledge, no other study dealt with the size of coral heads and its potential influence on geochemical signature over the biomineralized inorganic skeleton.

We tested Marshall and McCulloch's hypothesis on the Vanuatu corals. Kilbourne et al. (2004b) published a modern Vanuatu coral record with 65 years of monthly Sr/Ca data that varied with temperature. We sampled a small coral head (26 cm in diameter, 22 cm high) that was removed from the same island (assuming that Sr/Ca is spatially and temporally constant at the timescales studied). The difference between the mean is 0.096 mmol/mol for 3 years (Fig. 4). Reconstructing the SST based on the Kilbourne et al. (2004b) equation (0.05 mmol/mol of Sr/Ca per 1°C), the SST difference between the cores is 1.9°C, with the smaller head recording cooler temperatures. This is the opposite to what one can expect from the coral locations, as the small coral head lived in a more confine environment than the large coral head (bay vs. open water pass). This study tends to agree with Marshall and McCulloch's (2002) hypothesis regarding size effect on coral Sr/Ca composition.

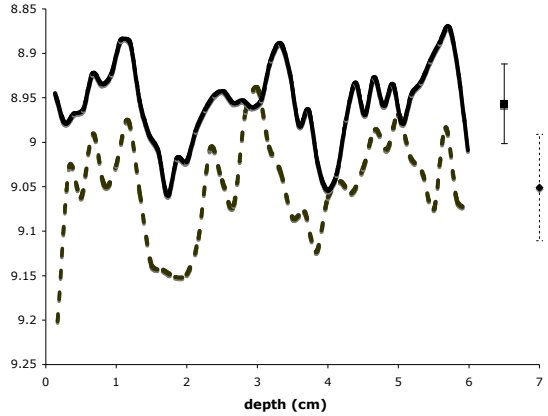

Figure 4: Comparison between Sr/Ca series from a small (dotted line) and a larger Vanuatu coral heads. Mean values are shown with one standard deviation. Sr/Ca scale is inverted to mimic SST.

Does age matter? The New Caledonia coral

Fig. 3 presents geochemical composition (Mg/Ca, Sr/Ca, δ^{13}C and δ^{18}O) of 25 samples from New Caledonia coral. The modern part (top slice) is compared to the basement (bottom slices).

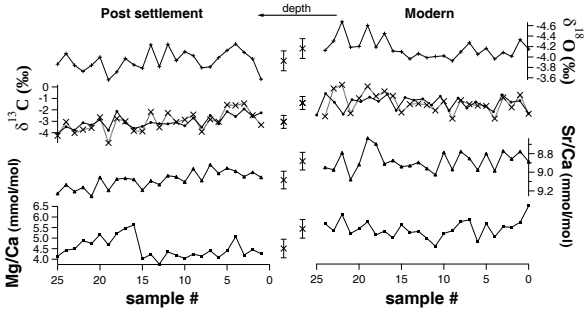

Figure 3: Geochemical data. From bottom to top: Mg/Ca, Sr/Ca, δ^{13}C and $\delta^{13}C_{transformed}$ to remove kinetic effect (crosses, see text for details) and δ^{18}O. The post-settlement period (bottom slices) is on the left and the modern period (top slice) is on the right side. Average values for each series are also shown with one standard deviation, in the center of the figure. 25 samples with half yearly resolution, i.e. ~12.5 years in total, are presented. Sr/Ca and δ^{18}O scales are inverted to mimic sea surface temperature variations.

Concerning the modern period, correlation coefficients between proxies (Table 1) are consistent with previous studies from New Caledonia (Stephans et al. 2004; Ourbak et al. 2006). Sr/Ca and δ^{18}O, anti-correlated to SST, are commonly used as

paleothermometers in corals. Mg/Ca varies with temperature (e.g. Mitsuguchi et al. 1996) which explains the negative correlation with Sr/Ca and $\delta^{18}O$ in Table 1.

The post-settlement period has a different geochemistry than the modern section (Fig. 3 and Table 1). For example, the post-settlement period records the Sr/Ca maximum and the $\delta^{13}C$ minimum of the New Caledonia data presented in Fig. 3 but also of the whole core (over 1000 samples), implying that this period was very different from the rest of the coral history. During the post-settlement period, Mg/Ca show no correlation with Sr/Ca and $\delta^{18}O$. Mg/Ca are on average lower during the post-settlement period vs. modern period (4.50 vs. 5.44 mmol/mol). On the contrary, Sr/Ca and $\delta^{18}O$ show higher averages during the post-settlement period (9.08 vs. 8.88 mmol/mol and -3.92 vs. -4.16‰, respectively). All these proxies vary principally with sea surface temperature and reveal cooler values for the bottom of the core. The average Mg/Ca difference between top and bottom, 0.94 mmol/mol, corresponds to a 4.3°C difference (using 0.218 mmol/mol per °C, Ourbak et al. (2006)). A 3.8°C SST variation is recorded in Sr/Ca (using a -0.0528 mmol/mol per °C (Ourbak 2006)) and $\delta^{18}O$ records a 1.5°C difference but this value could be subdued by the salinity influence on $\delta^{18}O$.

Although undated, based on extension rate and the total length of the core, we project the post settlement period ~7 centuries ago. A ~4°C is cooler than any similar record for the last millennium and would equate to the Younger Dryas 4.5°C cooling recorded in a Vanuatu coral (Corrège et al. 2004) (to compare with 1.4°C cooling during the Little Ice Age (1701-1761) period in New Caledonia (Corrège et al. 2001)). This would imply that temperature doesn't have the same effect on Sr, Mg and oxygen isotopes incorporation at the early stage of life of this coral compare to nowadays or that other parameters affect Sr/Ca, Mg/Ca and $\delta^{18}O$.

$\delta^{13}C$ is on average lower during the post-settlement period than the modern period (-3.05 vs. -1.4‰). One plausible factors causing $\delta^{13}C$ difference between New Caledonia slices could be the light-effect and its potential metabolic shift associated. According to several studies (see Ourbak et al submitted, and references therein) the ~7m difference between top and bottom slices could be sufficient to affect $\delta^{13}C$ signal by a 1.4‰ range as shown here (see depth-effects in the $\delta^{13}C$ composition e.g. McConnaughey 1989). This hypothesis alone is appealing but one should keep in mind that $\delta^{13}C$ in coral skeleton is difficult to interpret due to multiple affecting factors (Dissolved Inorganic Carbon, Photosynthesis/ Respiration rate, kinetic and metabolic factors etc). We also present $\delta^{13}C_{transformed}$. A mixture of kinetic isotope effects (resulting in isotopic depletion of carbon relative to isotopic equilibrium) and an equilibrium process control isotopic composition of corals. We applied a simple data transformation to correct for disequilibrium kinetic influences and to emphasize $\delta^{13}C$ metabolic signals only ($\delta^{13}C_{transformed} = \delta^{13}C_{original} - 3*(\delta^{18}O_{original} - \delta^{18}O_{average})$), see Heikoop et al. 2000). $\delta^{13}C_{transformed}$ show that kinetic effects at the early stage of life had the same range of effect on $\delta^{13}C$ signal than later in life (Fig. 3).

An explanation of the results presented here could be that ontogenic effects affect coral geochemical composition. Only a few studies have reported geochemical signature of the time of post-settlement of corals. In a very high resolution study (50 samples per year), Gagan et al. (1996) report on spawning events of two coral heads from two different reefs. A reproductively mature colony from Pandora Reef (6 years of growth at the top of a ~300 years old massive *Porites*) "revealed sharp ^{13}C enrichments closely corresponding to the time of the annual synchronized coral spawning event" and $\delta^{13}C$ and $\delta^{18}O$ signals are in phase. On the contrary, a small colony ~15 years old from Ningaloo Reef present no $\delta^{13}C$ annual variations for the first 6 years and when variations happen, $\delta^{13}C$ is out of phase with $\delta^{18}O$ signal. Gagan et al. (1996) suggest, "$\delta^{13}C$ signature is linked to the chemistry of the early reproductive patterns typical of juvenile massive corals". Thus monotonous $\delta^{13}C$ signature would correspond to years without reproduction, and subsequent shift to greater $\delta^{13}C$ variability would reflect the onset of "adolescence" of the coral, punctuated by brief annual reproductive cycles.

Even if our study misses the very first years of the post-settlement and our sampling resolution does not capture reproductive cycles, the ~2‰ $\delta^{13}C$ increase over the first 25 samples is similar to the one observed by Gagan et al (see Gagan et al 1996, Fig. 1B) concerning the differences between non-reproductive and reproductive years. Based on our preliminary results, we can anticipate that slice IP1/36 does not reflect environmental variability but rather is geochemically affected by early stage of growth and that Sr/Ca, Mg/Ca and $\delta^{18}O$, commonly used as SST proxy reveal erroneous reconstructed temperature.

Preliminary conclusions and implications for coral research.

We compared coral skeleton geochemical composition recorded by two different size Vanuatu corals and by the top and the basement of a New Caledonia core and find in both case large variations.

Mean Sr/Ca show a systematic shift in between small and larger Vanuatu corals. The Sr/Ca, Mg/Ca, $\delta^{18}O$ and $\delta^{13}C$ reveal anomalous values between New Caledonia core sections that are not due to diagenetic perturbations. We propose they are the results of ontogenic effects.

If one apply a calibration equation initiated from a large coral head to a small coral head, as it is often the case in fossils studies, the absolute temperature reconstruction could be biased by as much as 1.9°C based on Vanuatu example. Moreover, in a best-case scenario, when calibration is from the same core, ontogenic effects could alter the geochemistry at the youngest portions, thus introduce a bias in SST reconstruction, as demonstrated in the New Caledonia core.

These findings have important implications for paleoenvironmental studies, as in small coral colonies, as well as juvenile parts, one should be aware of the potential size/age effect and interpret Sr/Ca results in terms of relative changes rather than absolute temperature value. In other words, temperatures reconstructed from a fossil coral not collected in growth position, i.e. without indices of total length or age of the specimen could be biased if the coral has not reached maturity. Could we find a size threshold corresponding to sexual maturity for commonly used coral species? Can we quantify the ontogenic effect to discriminate against climatic factors affecting coral proxies? Numerous questions remain and further studies are needed to better understand biomineralization processes and how ontogenic factors affect the geochemical composition of coral skeleton.

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Opposed trend of skeletal carbon isotopic ratios found in two different coral species collected from the same site: Genus-dependent responses

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Abstract. For better understanding of carbon isotope systematic of coral skeleton, two different coral species growing next to each other, *Porites sp.* and *Platygyra ryukyuensis*, were collected at a site from Ishigaki Island, Japan, and analyzed for oxygen and carbon isotope ratios. Oxygen isotope ratios of both corals show seasonal variations well matching each other and that of observed sea surface temperatures. However, carbon isotope ratios were opposed in trends between two coral species. Carbon isotope ratios of *Porites* show a seasonal fluctuation matching that of solar radiation, while those of *Platygyra* are opposite in trend to that of solar radiation with weaker amplitude. Given that both coral colonies had been exposed to same environmental conditions during the growth period, these opposite trends should be attributed to different species-dependent vital response to the same environmental condition. A vector analysis indicates that seasonal variation of carbon isotope ratios are dominantly controlled by metabolic isotopic effect in both corals and also that the opposite trends in seasonal fluctuations of two coral species result from the opposite direction of metabolic isotope effect acting on two coral species. *Porites* shows a negative slope in a $\delta^{18}O$-$\delta^{13}C$ plot, in which seasonal shifts of $\delta^{18}O$ and $\delta^{13}C$ from colder to warmer season are explained with increased temperature and increased photosynthesis/respiration (P/R) ratios, indicating increased food-dependency on symbiotic algae (autotrophy) during warmer season. In contrast, *Platygyra* shows a positive slope in a $\delta^{18}O$-$\delta^{13}C$ plot, in which seasonal shift of $\delta^{13}C$ to summer is caused by decreased P/R ratios, indicative of reduced dependency on autotrophy during warmer season. This physiological characteristic of *Platygyra* might provide a tolerance to colder and weaker solar radiation environmental condition, which allows its wide distribution in middle latitude region.

Key words: coral skeleton, carbon isotope compositions, species dependency, *Porites*, *Favia*.

Introduction

Geochemical composition of reef-building coral skeleton has been a popular tool for high-resolution paleoclimatic reconstructions because coral skeleton shows systematic seasonal changes in isotopic and elemental compositions according to seasonal environmental changes. In particular, the stable oxygen isotopic values ($\delta^{18}Oc$) and Sr/Ca ratios of coral skeleton have proven to be reliable proxies for sea surface temperature (SST) and salinity (SSS) (Gagan et al. 2000). Boron isotopic composition, Cd/Ca, U/Ca ratios have been used as pH, upwelling, and temperature indicators, respectively (Pelejero et al. 2005; Reuer et al. 2003; Min et al. 1995).

Various controlling factors have been proposed for carbon isotopic value of coral skeleton ($\delta^{13}Cc$), including the $\delta^{13}C$ of dissolved inorganic carbon (DIC) in seawater (Nozaki et al. 1978; Swart et al. 1996), light intensity for photosynthesis of the zooxanthellate (Weber and Woodhead 1970; Swart 1983; Wellington and Dunbar 1995; Reynaud-Vaganay et al. 2001; Grottoli 2002; Asami et al. 2004; Shimamura et al. 2008), utilization and $\delta^{13}C$ of CO_2 originating from respiration (Swart et al. 2005), kinetic isotope effects (KIE) related to the coral calcification rate (McConnaughey 1989a,b; Omata et al. 2005; Suzuki et al. 2005), changes in the coral autotrophy/ heterotrophy diet (Carriquiry et al. 1994; Felis et al. 1998; Grottoli 2002), and coral spawning (Gagan et al. 1994, 1996). However, vital effects and physical controlling mechanisms of $\delta^{13}Cc$ are not fully understood yet. Because of it, the carbon isotopic value of coral skeleton ($\delta^{13}Cc$) has limited applicability in paleo-environmental reconstructions, other than chronology of long-lived coral records (e.g., Cole et al. 1993). Given that $\delta^{13}Cc$ is measured simultaneously with $\delta^{18}Oc$, shows seasonal variation as clearly as $\delta^{18}Oc$ does, and reflects both environmental and biological signals, it has great

potential as a powerful tool for paleo-environmental reconstructions.

For better understanding of controlling factor of δ^{13}Cc, two different coral species growing next to each other, *Porites sp.* and *Platygyra ryukyuensis*, were collected at a site from Ishigaki Island, Japan, and analyzed for δ^{13}Cc and δ^{18}Oc. The results were compared with observed environmental variables. *Platygyra* is one of hermatypic reef building massive corals that show a wide geographical distribution from tropics to higher latitude regions up to 35°N in the northwest Pacific (Yamano et al. 2004). Its geographical distribution extending to middle latitude region could allow us the development of a coral environmental proxy that can be used for middle latitude region. *Porites* was sampled together for the comparison with the results of *Platygyra* because it has provided reliable reconstructions of environments in tropical to subtropical regions of low latitude.

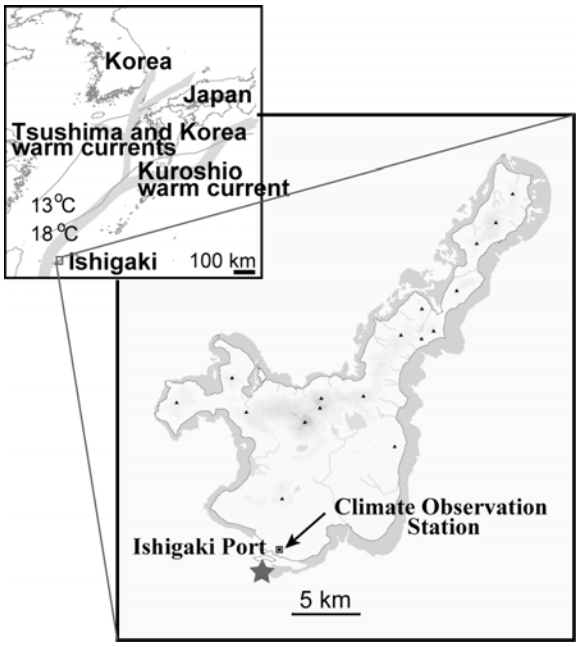

Figure 1: Location map showing the coral sampling sites (star mark) and climate observation station operated by Meteorological Agency of Japan.

Study site and method

Ishigaki Island is located in the southern part of Ryukyu Island Chain, Japan (Fig. 1). Two coral colonies, *Platygyra ryukyuensis* and *Porites spp*., each ~20cm in height, were collected in September, 2005 at 2 m water depth below mean sea level near the Ishigaki Port in the southern part of Ishigaki Island (Fig. 1). The coral collection site (24°25'N, 124°09'E) is about 30 m away from the seawall of the port, facing open ocean. The coral colonies had grown next to each other and thus had been exposed to the same environmental conditions (i.e. SST, SSS, light intensity, δ^{13}C of DIC, and etc.). SST and light intensity were observed at a climate observation station of Japan Meteorological Agency, located in the Ishigaki Port (Fig. 1). The highest light intensity were observed in June–July, whereas the highest SST is recorded in July–August during the study period

The collected coral samples were cut into 5 mm-thick slabs oriented parallel to the maximum growth axis. These slabs were cleaned with de-ionized water and X-rayed using an M-60 (SOFTEX Co). The X-radiographs of the coral slabs showed clear image of corallites, the skeleton of an individual polyp, and annual growth bands (Fig. 2). A continuous corallite along the major growth axis was selected from each slab for micro-sampling (Fig. 2).

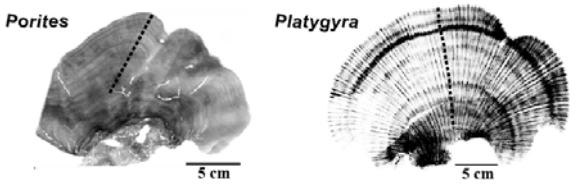

Figure 2: Positive prints of soft X-ray radiographs of coral slabs with micro- sampling transects (dashed lines).

Micro-sampling was carried out using a ledge micro-sampling technique (Gagan et al. 1994). For each sample, a 1-mm-thick and 3-mm-wide ledge was prepared along the selected corallite. The ledges were then ultrasonically cleaned with distilled water and dried in an oven at 40°C. Micro-sampling was undertaken on an accurate-distance (±10μm) movable table at a 200μm interval using a 0.8-mm-diameter end-mill drill bit mounted on a micro-milling machine. Such a sampling interval provides the time resolution of ~ 6 days for *Platygyra* and ~10 days for *Porites*, but the δ^{18}O and δ^{13}C measurement were undertaken at a time resolution of 25 and 20 days at peak areas, respectively. For *Porites*, sampling was carried out for one corallite that includes theca wall, columellae, and septa because of narrow width of corallite (av. 1mm). *Platygyra* was sampled only along the theca wall because of a wide corallite width (3 ~ 4.4mm) and absence of robust columellae and septa structures.

Powdered coral samples were reacted with 100% phosphoric acid at 70.0˚C in an automated individual-carbonate reaction device (Kiel Device) coupled with a Finnigan MAT 251 mass spectrometer. The resulting CO_2 gas was calibrated using NBS-19 standard. The oxygen isotopic ratio for the sample was reported relative to the Vienna Pee Dee Belemnite (V-PDB) in conventional delta notation. The precision for the analysis were 0.07‰ for both

$\delta^{18}O$ and $\delta^{13}C$ based on replicate measurement of NBS-19 standard (n=25).

Results

Both corals show a clear seasonal variation in $\delta^{18}Oc$ that agree with that of SST (Fig. 3). However, $\delta^{13}Cc$ shows different fluctuation pattern between *Platygyra* and *Porites* in magnitude and seasonality. $\delta^{13}Cc$ of *Porites* varies from 0 to -5‰ for the time period of 1995 to 2005 with a clear seasonality, higher values in summer and lower values in winter. This seasonal fluctuation pattern is in phase with that of solar radiation (Fig. 3). In contrast, *Platygyra* shows $\delta^{13}Cc$ values varying from 0 to -4‰ values with weaker seasonality that is opposed to that of *Porites*, higher in winter and lower in summer (Fig. 3). Since both corals had been exposed to the same environmental conditions during their growth period, these opposed trends should be attributed to the species-dependent vital factors, such as kinetic isotope effect due to different calcification rates between two species or different species-dependent physiological responses of two species to the same environmental condition. Seasonal extension rate and calcification rate, estimated based on $\delta^{18}Oc$ variation and x-ray photo analysis, do not show correlation with $\delta^{13}Cc$, which means these variations cannot be explained by KIE.

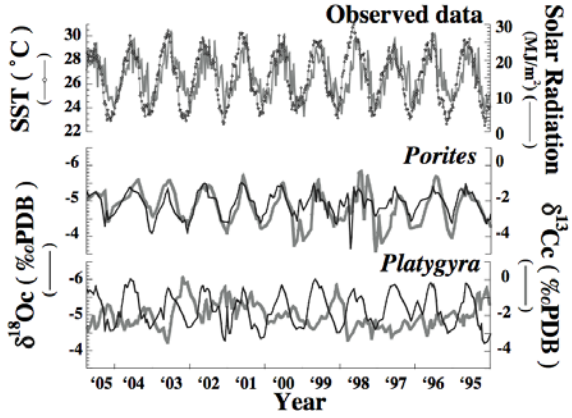

Figure 3: Temporal variation of the observed sea surface temperature and solar radiation, and the measured oxygen and carbon isotope ratios of *Porites* and *Platygyra* during the growth period

Discussion

In order to understand controlling factors responsible for the opposed seasonal variation in the carbon isotope ratios of two coral species, we carried out a vector analysis similar as in McConnaughey (1989) and Omata *et al.* (2005). In this approach, variation of $\delta^{13}Cc$ and $\delta^{18}Oc$ is explained with sum of three vectors with magnitude and direction: 1) temperature dependency of $\delta^{18}Oc$; 2) metabolic isotope effects (MIE) controlled by photosynthesis of symbiotic algae and respiration; and 3) KIE related to calcification rate of skeleton (Fig. 4).

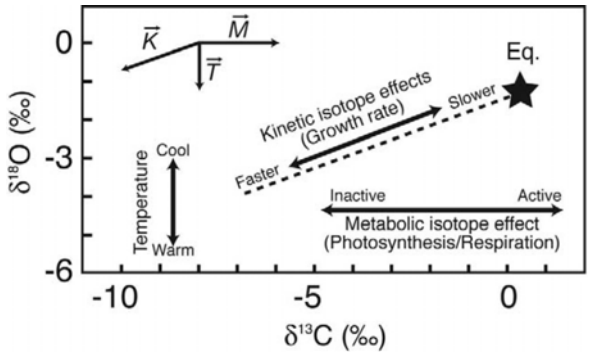

Figure 4: Schematic diagram showing the effects of temperature, kinetic isotope effect and metabolic isotope effect on $\delta^{13}C$ and $\delta^{18}O$, adapted from Omata *et al*. (2005). Eq.: isotopic composition of biogenic carbonate precipitated under isotopic equilibrium; K, M, and T: vectors representing the magnitude and direction of kinetic isotope effect, metabolic isotope effect, and temperature, respectively.

We plotted the determined $\delta^{18}Oc$ and $\delta^{13}Cc$ values in a $\delta^{18}Oc$-$\delta^{13}Cc$ plane on a yearly base (Fig. 5 as an example). In these plots, *Porites* always shows negative slope for all the years from 1995 to 2005 (Fig. 5), in which seasonal shift of $\delta^{18}Oc$ and $\delta^{13}Cc$ from winter to summer can be explained by the sum of negative vector of temperature and positive vector of MIE with a little change in KIE. Negative vector of temperature indicates increase in temperature and positive vector of MIE represents increase in the ratio of photosynthesis to respiration (P/R) toward warmer season. Active photosynthesis of symbiotic algae during warmer season is responsible for the positive vector of MIE.

For the result of *Platygyra,* the slope against whole values showed positive slope in a $\delta^{18}Oc$-$\delta^{13}Cc$ plot for all the analyzed year (Fig. 5). For each vector components, little variation in magnitude of KIE between seasons as in *Porites,* MIE causes negative $\delta^{13}Cc$ shifts because of the negative vector, and it is opposite to that in *Porites*. It means high P/R ratios during winter and non-active photosynthesis by symbiotic algae during warm season or much higher dependency of zooplankton. Also in general, MIE variation is much greater than KIE.

In contrast, *Platygyra,* shows positive slope in a $\delta^{18}Oc$-$\delta^{13}Cc$ plot (Fig. 5), in which seasonal shift of $\delta^{18}Oc$ and $\delta^{13}Cc$ from winter to summer is now explained by the sum of negative vector of temperature, same as in *Porites*, and negative vector of MIE, opposite to that of *Porites* (Fig. 5). As in *Porites*, KIE has negligible effect on seasonal variation of $\delta^{18}Oc$ and $\delta^{13}Cc$ (Fig. 5). Negative vector

of MIE indicates increased importance of respiration relative to photosynthesis during warmer season in *Platygyra*. It can not be conclusive, but might suggest that *Platygyra* is less dependent on autotrophy (food source from symbiotic algae) during warmer season. This physiological characteristic of *Platygyra* might provide a tolerance to colder and weaker solar radiation, which allows wide distribution of *Platygyra* in middle latitude region.

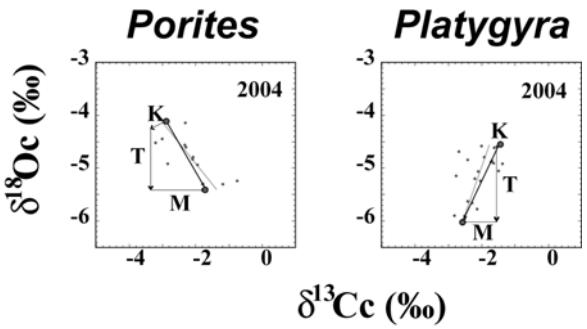

Figure 5: Vector approaches to explain seasonal changes of carbon and oxygen isotopic ratios of the studied corals. K, M, and *T* show the direction and magnitude of the kinetic, metabolic, and temperature effects, respectively.

Conclusions

Oxygen isotopic ratios of *Porites sp.* and *Platygyra ryukyuensis* that had grown next to each other show seasonal variations well matching each other and that of observed sea surface temperatures. However, carbon isotope ratios were opposed in trend between *Porites* and *Platygyra*. Carbon isotope ratios of *Porites* showed a seasonal fluctuation matching that of solar radiation, while those of *Platygyra* were opposite in trend to those of solar radiation and *Porites*. Given that both coral colonies had been exposed same environmental conditions, these opposed trends are likely attributed to species-dependent vital factors rather than environments.

Vector analysis indicates that seasonal variation of δ^{13}Cc is dominantly controlled by MIE over KIE in both corals. *Porites* shows a negative slope in a δ^{18}O-δ^{13}C plot, in which seasonal shifts of δ^{18}O and δ^{13}C from colder to warmer season are explained by increased temperature and photosynthesis/respiration (P/R) ratios, indicating more dependency on food from symbiotic algae during warmer season. In contrast, *Platygyra* shows a negative slope in a δ^{18}O-δ^{13}C plot and decreased P/R ratios during warmer season, indicative of reduced dependency on autotrophy during warmer season.

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U/Ca as a possible proxy of carbonate system in coral reef

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Abstract. Uranium incorporation into coral skeleton of *Goniastrea aspera* was investigated in the laboratory incubation for 8 hours under controlled pCO_2 and temperature. Seawater parameters such as pH and alkalinity as well as calcium and uranium concentrations were measured every 2 hours. pH increased progressively from 8.17 at initial to 8.54 at the end. In contrast, alkalinity significantly decreased from 2292 to 1467 mmol/kg. The apparent distribution coefficients of uranium, λUO_2, between *Goniastrea aspera* skeleton and seawater were obtained to be 2.7 to 0.7. The data were compiled with those of other studies and the overall data illustrate a negative correlation between λUO_2 and $a CO_3^{2-}$ with the slope of −0.75. It is suggested that variation of carbonate ion activity (i.e. carbonate system) controls the distribution coefficient of uranium and hence coral U/Ca. The effect of uranyl carbonate complexes formation on uranium incorporation is discussed.

Key words: Uranium, distribution coefficient, proxy, carbonate system

Introduction

Carbon dioxide is an extremely important greenhouse gas in the atmosphere, playing a significant role in controlling the Earth's surface temperature. The atmospheric carbon dioxide level has already increased from the concentration of 280 ppm in the pre-industrial age to ~380 ppm today, and it is predicted to reach ~560 ppm before the end of the 21st century (Prentice et al. 2000). Due to the capability of the ocean as a significant reservoir of carbon dioxide from atmosphere, such increases of atmospheric carbon dioxide have some impacts to either physical or chemical properties of oceanic seawater. One of the impacts is the decline of CO_3^{2-} concentration in seawater which is in advance modifying the carbonate system of the surface ocean and eventually affects the metabolism, such as photosynthesis and calcification of marine organisms.

During calcification process of coral, some metal ions are able to be incorporated into carbonate lattice through the ion exchange mechanism between metal and calcium ions. Distribution coefficient of metal (D_{Me}) between coral skeleton and seawater is defined as follows (McIntire 1963; Shen and Boyle 1986; Lea and Spero, 1992):

$$D_{Me} = \frac{[Me/Ca]_{coral}}{[Me/Ca]_{seawater}} \quad (1)$$

For the case of uranium, Shen and Dunbar (1995) reported that the range in the coral/seawater uranium distribution coefficient is 0.8−1.0 once uranium concentration is 13.4 nM (Chen et al. 1986). Furthermore, several studies have been measured the uranium distribution coefficient of 0.5−2.0 which is depended on the coral species (Veeh and Turekian 1968; Schroeder et al. 1970; Thompson and Livingston 1970; Flor and Moore 1977; Swart and Hubbard 1982). The measurement of the distribution coefficient of uranium between carbonate and solution is a useful tool for the understanding of the factors controlling the uranium contents of marine calcareous sediments (Kitano and Oomori 1971; Min et al. 1995).

It is well known that uranium in seawater exists not only a uranyl ion (UO_2^{2+}) (Ku et al. 1977) but also anion complexes with carbonate (i.e. $UO_2CO_3^0$, $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$) (Grenthe et al. 1992; Shen and Dunbar 1995). It is believed that the formation of such uranyl carbonate complexes is influenced by the carbonate system of seawater. One of parameters to control carbonate system is the carbonate ion activity. Based on this fact, the incorporation mechanism of uranium into coral skeletons is considered as UO_2^{2+} (cation mode) (Kitano and Oomori 1971; Broecker and Peng 1982) or $UO_2(CO_3)_2^{2-}$ (anion mode) (Swart and Hubbard 1982; Shen and Dunbar 1995). Such mechanism remains unclear and nowadays still in debate. In this study, the incubation experiment of coral *Goniastrea aspera* was done to test that carbonate ion activity of solution affects the uranium incorporation into coral

skeleton. The incorporation mode (i.e. cation versus anion mode) of uranium is discussed.

Material and Methods

Coral incubation

We used a coral colony of *Goniastrea aspera* for incubation experiment. Fig. 1 shows a schematic picture of coral incubation. We incubated *G. aspera* (18×26×15 cm^3) in the seawater tank (27×38×24 cm^3) under direct sunlight for 8 hours, and collected seawater samples in the polyethylene bottles every two hours. Photon flux was measured to be 200−800μmol/m^2/s and temperature of the system was 24.5±1.5°C during incubation.

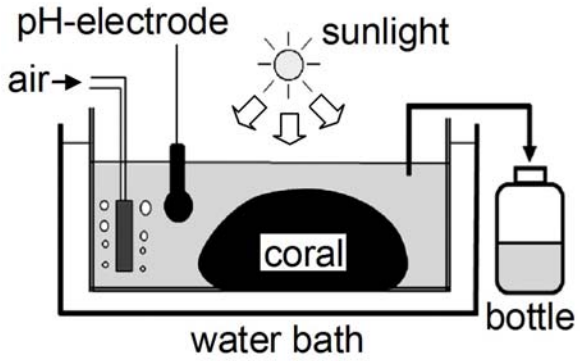

Figure 1: Schematic picture of incubation experiment.

Seawater analysis

The seawater pH was measured immediately after collecting seawater sample. The collected seawater was filtered on a Millipore HA (0.45 μm pore size) filter, and analyzed for alkalinity. The remaining seawater sample was preserved by adding conc. HCl to pH=2, and then was stored for subsequent analysis of total Ca and U.

The pH of seawater was measured by pH meter (Orion 290 A+Thermo, Japan). In each set of measurement, pH meter was calibrated using NBS-scaled buffer solutions (TOA, Golden buffer) at pH=7 (6.863) and pH=4 (4.006) at 25°C. Reproducibility of the measurements (n=11) was found to be ±0.003 pH unit (1σ).

Total alkalinity was measured potentiometrically using an auto titration system (TIM 860 Radiometer, TitraLab, France). Reproducibility of the measurements (n=9) was found to be ±3 μmol/kg (1σ).

Water salinity was measured by a salinometer (Model 8410A, Canada) using the reference standard seawater of IAPSO (K_{15}= 0.99987, S=34.995). Reproducibility of the measurements (n=11) was ±0.001‰ (1σ).

Calcium in seawater samples were determined by EGTA titration method (Kanamori and Ikegami 1980) using an auto titration system (TIM 865 Radiometer, TitraLab, France) equipped with Ca selective electrode. The equivalence point for each titration was obtained by a Gran's plot method. Reproducibility of the measurements (n=11) was ±0.01 mmol/l (1σ).

For the determination of uranium in solution, 15 ml of seawater sample in 30 ml of conc. HCl was passed firstly to anion exchange resin (Dowex 1×8, 100~200 mesh) column to separate uranium from matrix elements. After washed with 8N HCl, sample was eluted by 25 ml of 0.1 N HCl. Recovery of the separation was ~98% and reproducibility of the measurement of 3 ppb uranium was ±0.08 ppb (n=10; 1σ). The eluted-seawater samples were then introduced to ICP-MS (Hewlett Packard, HP 4500) with Ar-H_2 gas carrier for uranium quantification.

The apparent distribution coefficient of uranium (λ_{UO_2}) associated with CO_3^{2-} ion activity during experiment was calculated with the Doerner-Hoskins' equation (Doerner and Hoskins 1925; Kitano et al. 1968):

$$\lambda_{UO_2} = \frac{\log(M_{UO_2,initial} / M_{UO_2,final})}{\log(M_{Ca,initial} / M_{Ca,final})} \quad (2)$$

where $M_{i,initial}$ denotes the molar concentration of i species dissolved in an incubated seawater at t = 0 and $M_{i,final}$ denotes i species at a given time of reaction.

Results

The temperature ranged from 23−26°C during incubation was a suitable circumstance for metabolism of coral (Coles and Jokiel 1977). Parameters such as pH and alkalinity with respect to coral metabolism changed during experiment. Fig. 2 shows the measurements of pH, alkalinity and salinity of seawater every 2 hours of incubation. pH value ranged from 8.17 at initial to 8.54 at the end with average value of 8.43 (Fig. 2a). The calcification process in which the calcium carbonate skeleton was formed diminished carbonate ions from incubated seawater. Such process decreased the alkalinity from 2292 to 1467 μmol/kg (Fig. 2b). However, for the case of salinity, it slightly increased from 34.6 to 35‰ (Fig. 2c), indicating to a slight evaporation during experiment. Shen and Dunbar (1995) suggests that U/Ca ratio in corals is affected by salinity changes in areas subject to significant rainfall or run-off, however, the salinity changes in this study are on a small scale to have impact to the coral U/Ca.

It is considered that the difference in calcium concentration in solution between initial (t=0) and every two hours of incubation time refers to the

amount of cumulative precipitated $CaCO_3$ during the time (Fig. 3). Therefore, due to calcification process, the cumulative amount of $CaCO_3$ increased from 0 at initial to 0.41 mmol/l at the end of experiment. Moreover, during calcification process uranium was incorporated into coral skeleton as indicated by the enhancement of precipitated uranium from 0 to 0.4 nmol/l (Fig. 3b).

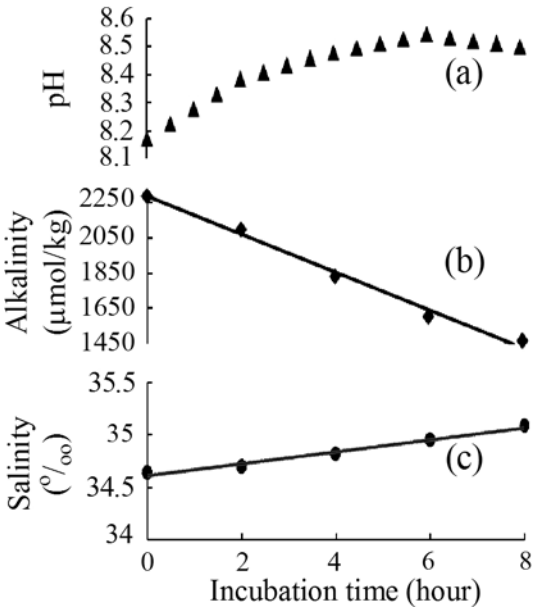

Figure 2: pH, alkalinity and salinity of seawater during experiment.

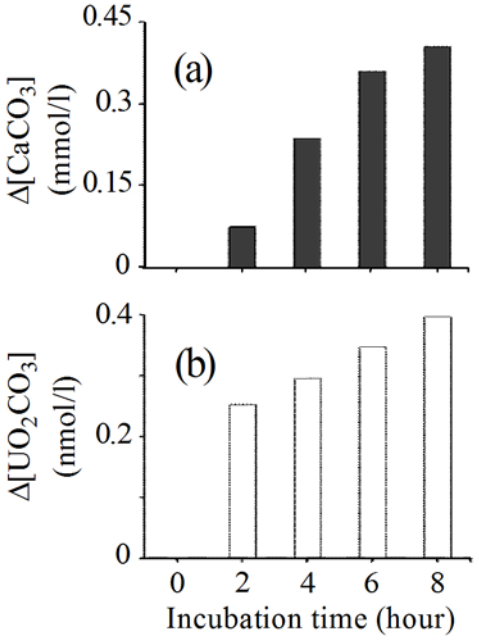

Figure 3: Amount of cumulative precipitation of $CaCO_3$ and UO_2CO_3 every two hours of incubation time.

Fig. 4 shows the relationship between apparent distribution coefficient of uranium and the carbonate ion activity during experiment. In the present study, distribution coefficient decreased from 2.7 to 0.7 as the carbonate ion activity declined from 5 to 4.4 μmol/l. The distribution coefficient data obtained from different coral species as well as inorganic precipitation experiment are also plotted in Fig. 4. The compiled-data reveal a trend of negative correlation between distribution coefficients of uranium and carbonate ion activities with the slope of –0.75.

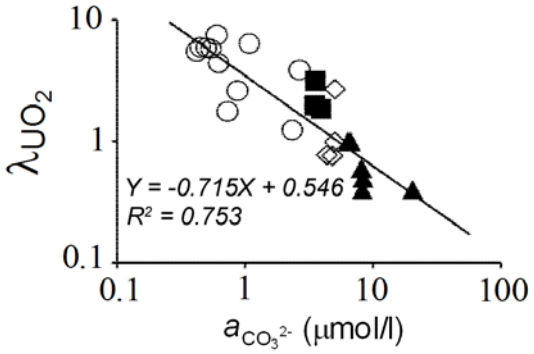

Figure 4: Distribution coefficient of uranium (λUO_2) plotted against the carbonate ion activity (aCO_3^{2-}). ○ = Takaesu, 2008 (Porites sp); ■ = Yoshida, 2000 (Fungia sp); ◇ = this study (*Goniastrea aspera*); ▲ = Kitano and Oomori, 1971 (Inorganic precipitation). The slope of regression line = –0.715.

Discussion

Incorporation of uranium into coral (*Goniastrea aspera*) skeleton under carbonate system change has been a focal point of this study. Fig. 4 shows that the value of the apparent distribution coefficient of uranium (λUO_2) decreased as the carbonate ion activity increased. This phenomenon is considered to be associated with the evidence that uranyl ions form complexes with carbonate ions in solution (Grenthe et al. 1992; Turner et al. 1981). Degree of complex formation of uranyl carbonate increases with increasing carbonate ion activity. Therefore, with increasing of carbonate ion activity, the activity of free uranyl ion dissolved in solution decreases and the distribution coefficient value should decrease (Kitano and Oomori 1971). Speciation of uranyl carbonate complexes in solution is important to discuss the incorporation mechanism of uranium into coral skeleton.

Turner et al. (1981) measured the stability constant (K) for the formation of carbonate species at T=25°C, P=1 atm, I=0.7, as follows:

$UO_2^{2+} + CO_3^{2-} \Leftrightarrow UO_2CO_3^{0}$; $K_1 = 10^{6.02}$

$UO_2^{2+} + 2CO_3^{2-} \Leftrightarrow UO_2(CO_3)_2^{2-}$; $K_2 = 10^{15.07}$

$UO_2^{2+} + 3CO_3^{2-} \Leftrightarrow UO_2(CO_3)_3^{4-}$; $K_3 = 10^{20.19}$

As far as the incorporation of uranium into coral skeleton is concerned, the species being incorporated can be either UO_2^{2+} (cation) or $UO_2(CO_3)_2^{2-}$ (anion) if the incorporation follows the ion exchange process stoichiometrically.

Cation hypothesis

Reaction: $CaCO_3 + UO_2^{2+} \Leftrightarrow UO_2CO_3 + Ca^{2+}$

In this system, a uranyl ion (charge = +2) in solution replaces a similar charge of calcium in solid ($CaCO_3$) phase. The distribution coefficient of uranium (K_{UO_2}) is given as follows:

$$K_{UO_2} = \frac{[UO_2CO_3]}{[CaCO_3]} \cdot \frac{[Ca^{2+}]}{[UO_2^{2+}]} \quad (3)$$

Theoretically, the apparent distribution coefficient of uranium is written (Kitano and Oomori 1971):

$$K_{UO_2} = \frac{1}{1+\alpha} \cdot K^{T}_{UO_2} \cdot \frac{\lambda^{S}_{CaCO_3}}{\lambda^{S}_{UO_2CO_3}} \cdot \frac{\gamma^{L}_{UO_2^{2+}}}{\gamma^{L}_{Ca^{2+}}} , \quad (4)$$

Where γ^L and λ^S denote the activity coefficients of ion species in solution and $MeCO_3$ in carbonate phase respectively, $K^{T}_{UO_2}$ is thermodynamic distribution coefficient of uranium. When $\lambda^{S}_{CaCO_3}$, $\lambda^{S}_{UO_2CO_3}$ and $\gamma^{L}_{UO_2^{2+}} / \gamma^{L}_{Ca^{2+}}$ are taken as unity, K_{UO_2} is depended on α. Since total uranium dissolved in solution ($M^{L}_{UO_2,T}$) is:

$$M^{T}_{UO_2,T} = M^{L}_{UO_2^{2+}} + M^{L}_{UO_2CO_3^0} + M^{L}_{UO_2(CO_3)_2^{2-}} + M^{L}_{UO_2(CO_3)_3^{4-}}$$
$$= (1+\alpha) M^{L}_{UO_2^{2+}} , \quad (5)$$

where, the value of α is given:

$$\alpha = K_1 \cdot a^{L}_{CO_3^{2-}} + K_2 \cdot (a^{L}_{CO_3^{2-}})^2 + K_3 \cdot (a^{L}_{CO_3^{2-}})^3 \quad (6)$$

Then, equation (4) can be re-written:

$$K_{UO_2} \cong \frac{1}{1 + \{K_1 \cdot a^{L}_{CO_3^{2-}} + K_2 \cdot (a^{L}_{CO_3^{2-}})^2 + K_3 \cdot (a^{L}_{CO_3^{2-}})^3\}} \cdot K^{T}_{UO_2} \quad (7)$$

It is seen that the distribution coefficient of uranium, therefore, is a function of carbonate ion activity.

Based on the equation (7), the relation between K_{UO_2} and $a^{L}_{CO_3^{2-}}$ shows a negative correlation with the slope = −1 when the value of $\alpha = K_1 \cdot a^{L}_{CO_3^{2-}}$ or $UO_2CO_3^0$ is a dominant species in solution. Moreover, the slope = −2 or −3 is produced in case of $UO_2(CO_3)_2^{2-}$ or $UO_2(CO_3)_3^{4-}$ is dominant, respectively, in solution.

We superimpose the modeled slopes of −1 and −2 from equation (7) over the reported distribution coefficient values (Fig. 4) and is shown in Fig. 5. The compiled-data of distribution coefficients seem in agreement with the slope = −1. It is suggested that $UO_2CO_3^0$ maybe a main species in incubated-seawater when we consider that uranium incorporation follows a cation mode. However, the data from individual experiments are somewhat scattered. The trend is still un-clarified yet, hence advanced investigation is necessary for clarifying the trend.

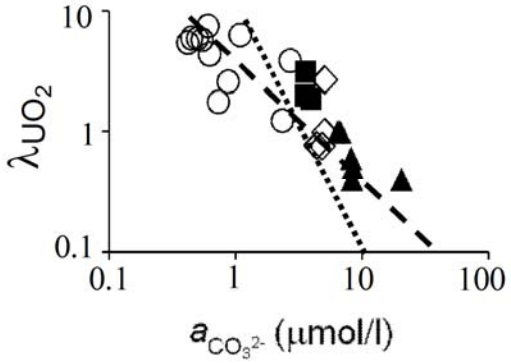

Figure 5: Distribution coefficient of uranium (λUO_2) plotted against the carbonate ion activity (aCO_3^{2-}). The slopes of ..... = −2, and — — = −1 (see text).

Anion hypothesis

Reaction: $CaCO_3 + UO_2(CO_3)_2^{2-} \Leftrightarrow CaUO_2(CO_3)_2 + CO_3^{2-}$

A uranyl carbonate ion (charge = −2) from solution substitutes for a same charge of carbonate ion from $CaCO_3$ phase. In this system, contribution from UO_2^{2+} is neglected and $M^{L}_{UO_2,T}$ is written:

$$M^{T}_{UO_2,T} = M^{L}_{UO_2CO_3^0} + M^{L}_{UO_2(CO_3)_2^{2-}} + M^{L}_{UO_2(CO_3)_3^{4-}}$$
$$= M^{L}_{UO_2(CO_3)_2^{2-}} \cdot \left\{ \frac{1}{\beta_2 \cdot a^{L}_{CO_3^{2-}}} + 1 + \beta_3 \cdot a^{L}_{CO_3^{2-}} \right\} \quad (8)$$

where β_2 denotes a stability constant of $UO_2(CO_3)_2^{2-}$ formation from $UO_2CO_3^0$ and β_2 represents a stability constant of $UO_2(CO_3)_3^{4-}$ formation from $UO_2(CO_3)_2^{2-}$. From equation (8), the abundance of $M^{L}_{UO_2(CO_3)_2^{2-}}$ relatives to the total dissolved uranium is:

$$\frac{M^{L}_{UO_2(CO_3)_2^{2-}}}{M^{L}_{UO_2,T}} = \frac{1}{\left\{ \frac{1}{\beta_2 \cdot a^{L}_{CO_3^{2-}}} + 1 + \beta_3 \cdot a^{L}_{CO_3^{2-}} \right\}} \quad (9)$$

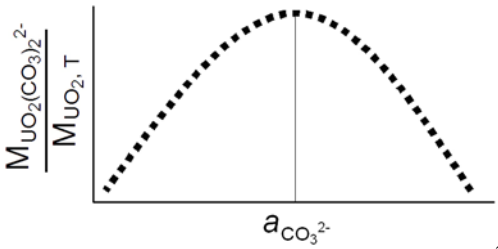

Figure 6: The relative abundance of $UO_2(CO_3)_2^{2-}$ plotted theoretically against the carbonate ion activity (aCO_3^{2-}).

Fig. 6 illustrates a conceptual plot of the relation between relative abundance of $UO_2(CO_3)_2^{2-}$ and carbonate ion activity in the solution. Vertical line in the middle denotes the critical value of carbonate ion activity. Around this vertical line, $UO_2(CO_3)_2^{2-}$ is considered to be a dominant species in solution. Relative abundance of $UO_2(CO_3)_2^{2-}$ is controlled by the equilibrium relation with $UO_2CO_3^0$ at below (i.e. slope = 1) or $UO_2(CO_3)_3^{4-}$ at above the critical value (i.e. slope = –1).

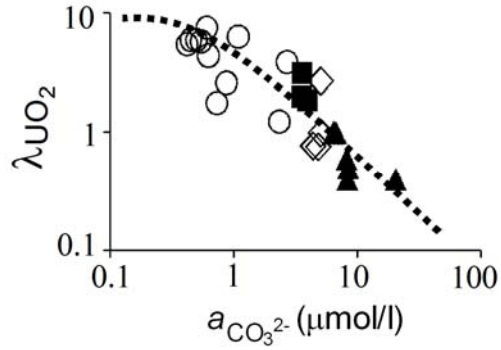

Figure 7: Distribution coefficient of uranium (λUO_2) plotted against the carbonate ion activity (aCO_3^{2-}). The slope of ..... = –1 (see text).

We superimpose the area of slope = –1 of Fig. 6 on the distribution coefficient values (Fig. 4) and is shown in Fig. 7. The entire data of the distribution coefficient of uranium lie within the slope = –1. It may be possible that $UO_2(CO_3)_3^{4-}$ is a dominant species in incubated-seawater when we consider that uranium incorporation as an anion mode.

At present, mode of uranium incorporation into coral skeletons remains unclear. Both candidates (i.e. cation and anion modes) seem valid, yet debatable. More detail studies on the distribution coefficient of uranium are significantly required to provide precise data for clarifying the mode of incorporation.

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Using nearshore macrobenthos as environmental indicators adjacent to a major navigational inlet: Port Everglades inlet, Florida

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Abstract. The reefs off Broward County, Florida, USA, lie near the northernmost limits of tropical coral reefs, are non-accreting, and have long been affected by human influences including land-based sources of pollution. Port Everglades, a major industrial shipping port, is a likely source of many anthropogenic contaminants, which are discharged in an effluent plume that sweeps over the coastal reef. The results of two nearshore reef studies were examined here to determine if the inlet effluent plume produces a water quality gradient and associated biological gradient, and if any biological indicators of water quality can be determined. Macrobenthos cover at 33 sites was assessed from Port Everglades inlet south to the Broward County line. Results showed macroalgae abundance significantly increases with proximity to Port Everglades inlet. In addition, water quality data clearly illustrate a low-salinity wedge being discharged from the inlet at low tide, as well as increased levels of nutrients around the mouth of the inlet. These results suggest that Port Everglades is a probable source of coastal pollution that may be causing localized increases in algal abundance that can be detrimental to the benthic ecology of the surrounding nearshore reef.

Key words: land-based pollution, effluent, indicators, coral reefs

Introduction

Coral reefs are used around the world for recreation, food, a source of income, and storm protection. Our lack of understanding of reef ecology and the effects humans have on reefs has taken a toll on these diverse ecosystems. In order to conserve remaining reef systems, proper sensitive and quantifiable biological indicators need to be developed for detection of impacts to reef systems, which can be used on a wide scale.

Monitoring and assessment tools that diagnose coral reef community responses to threats are critical to the management of these ecosystems. Corals themselves are extremely sensitive animals and require a narrow range of environmental conditions in order to thrive. However, corals are slow-growing and may take years to show damage from pollutants. Furthermore, coral growth rates themselves are poor indicators of reef health because growth rates often initially increase with nutrient dumping and land run-off (Koop et al. 2001; Wittenberg and Hunte 1992). Thus, other, more dynamic, taxonomic groups with shorter turnover times, e.g., algae and sponges, may express the effects of pollutants on a reef site in a time frame more feasible for study.

Areas of high nutrient addition often experience increased algal growth rates, which can be detrimental to corals, either by competition for growth and recruitment space or by blocking vital sunlight from reaching the coral (Marszalek 1981; Steneck 1997). High sedimentation, eutrophication, and overfishing of reefs may also reduce the settlement and recruitment of grazers, thereby promoting higher algal cover (Wittenberg and Hunte, 1992; Richmond et al. 2007). Reefs degraded by nutrient over-enrichment often exhibit "phase shifts" from abundant coral to abundant macroalgae (McCrook 1999; Szmant 2002). These observations may be used as early warning signals of reef health degradation.

Heterotrophic sponges may also represent an early warning signal for organic pollution of coral reefs (Wilkinson and Cheshire 1990). Evidence exists that sponge biomass is higher in nutrient-rich waters compared to oligotrophic waters (Wilkinson 1987; Wilkinson and Cheshire 1990). Tomascik and Sander (1985) found the east coast of Barbados, which experiences strong oceanic influences, has a significantly lower biomass of sponges compared to the west side of the island, which is exposed to high levels of nutrients from anthropogenic discharge. Aerts (1998) found that sponges overgrow corals to a greater degree where coral cover is low, suggesting that dying or degraded reefs with reduced living coral

cover may be susceptible to increased overgrowth by sponges. These studies suggests that sponge abundance and biomass are related to nutrient availability, water quality, and overall reef health, and have implications for coral reef impact studies and monitoring.

In Broward County, Florida, USA, Port Everglades may be a source of many anthropogenic contaminants, including nutrients and other pollutants carried in freshwater from coastal runoff, which extend in an ebb-tidal plume that sweeps over the adjacent coastal reef (Fig. 1). What direct effects these contaminants may have on adjacent reef life is hypothesized; however, direct studies on the effects of effluent from Port Everglades inlet are limited. This paper examines macrobenthic data collected from two nearshore community assessment studies in Broward County. These data are used to evaluate the effects of proximity of reef biota to a major navigational inlet and identify potential bioindicators of stress on the reef system.

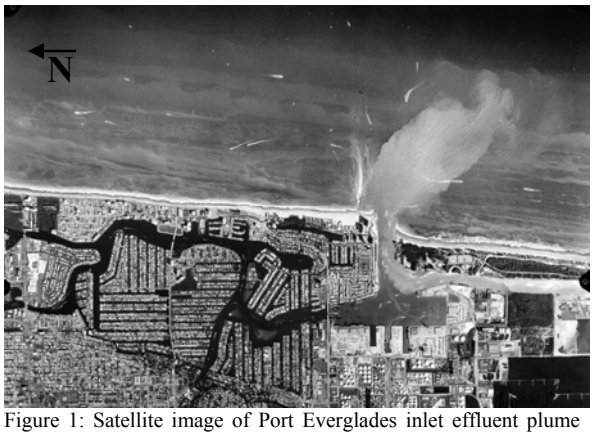

Figure 1: Satellite image of Port Everglades inlet effluent plume (image courtesy of Richard Dodge, National Coral Reef Institute).

Materials and Methods

Monitoring design

Results from two separate studies were combined. The first study (Study 1, Craft 2006) assessed the percent cover of macroalgae and sponge at seven nearshore sites immediately south of Port Everglades inlet**.** Preliminary results from Study 1 indicated that coral abundance within the study area is highly variable, and primarily dictated by variations in substrate type and availability rather than any existing water quality gradients; thus, other, more dynamic taxonomic groups, e.g. macroalgae and sponges, are examined here. Site placement was based on prior water quality surveys and thermograph stations used by Broward County Environmental Protection Department (BCEPD) (BCEPD, 2007). Sites were numbered from south to north, starting at BCEPD Thermograph Station JUL6. Each site consisted of three shore-parallel 30-m transects. A $0.25m^2$ x $0.25m^2$ quadrat was placed every other meter for a total of 45 quadrats per site, and 2.81 m^2 of area surveyed.

Water quality parameters were also measured during Study 1. A YSI data-sonde was used to measure specific conductivity (as a proxy for salinity). The probe was lowered haphazardly within the inlet plume over the general study area. Vertical readings were taken at these locations with the sonde, from surface to bottom in order to observe changes in specific conductivity with depth, implying a gradation in salinity with depth. Water samples were also taken from select areas across the influence of the plume and analyzed for chlorophyll *a* and nutrients: total phosphorus, nitrites and nitrates (NO_2+NO_3), and Total Kjeldahl nitrogen (TKN).

The second study (Study 2) was conducted as a pre-construction survey for the Segment III Broward County Beach Renourishment Project. Twenty-one transects were surveyed south of Port Everglades inlet to the southern Broward County boundary using the Benthic Assessment for Marginal Reefs (BEAMR) method (Lybolt and Baron 2006). A $0.5m^2$ x $0.5m^2$ quadrat was placed every 2.5 meters along a 30-m transect for a total of 12 quadrats per transect, and 3.0 m^2 of area surveyed. Sponge and macroalgae percent cover data were extracted from this data set and combined with data from Study 1 for analysis.

Data analysis

Benthic coverage data were first standardized for amount of hardbottom sampled (i.e. eliminating sediment cover). Data were then fourth-root transformed and compared, using linear regression, against distance from the inlet (latitude; dd.ddd) to determine if trends exist relative to the location of Port Everglades inlet.

Linear regression was also used to examine specific conductivity relative to distance from the inlet, and plotted against latitude (dd.ddd) and depth (m) to create vertical and surface-planar descriptions of the inlet plume and associated salinity "wedge". Chlorophyll *a* measured from grab samples was examined against specific conductivity in the same manner.

Results

Benthic cover

After standardizing for amount of hardbottom sampled, macroalgae cover ranged from 1.4% to 54.0%. Standardized sponge cover ranged from 0.1% to 2.7% (Table 1).

Table 1. Macroalgae and sponge cover (percent) standardized for amount of hardbottom sampled.

Study	Site	Latitude	Standardized Macroalgae	Standardized Sponge
2	88a	26.0868	27.9	1.1
1	7	26.0819	41.4	0.5
1	6	26.0813	33.6	0.6
2	90a	26.0813	27.4	0.5
1	5	26.0808	8.4	0.2
1	4	26.0803	8.3	0.4
1	3	26.0798	3.4	0.3
1	2	26.0793	9.1	0.4
1	1	26.0788	8.0	0.1
2	96a	26.0649	3.7	0.7
2	98a	26.0593	39.2	0.1
2	99a	26.0566	38.7	0.8
2	99b	26.0552	5.0	0.3
2	100a	26.0540	3.4	2.1
2	100b	26.0520	7.1	2.7
2	101a	26.0509	3.9	1.0
2	104a	26.0428	11.3	1.8
2	104b	26.0411	2.6	0.7
2	105b	26.0383	3.3	1.9
2	106a	26.0369	54.0	0.0
2	108a	26.0316	6.7	0.4
2	110a	26.0258	18.8	0.4
2	113a	26.0176	14.4	0.4
2	116a	26.0093	13.9	0.6
2	119a	26.0008	5.2	1.2
2	120a	25.9977	1.7	0.7
2	120b	25.9962	1.4	0.6
2	121b	25.9935	2.2	0.5
2	122a	25.9921	1.9	0.7
2	123a	25.9891	11.5	0.5
2	125a	25.9835	2.1	0.6
2	126b	25.9790	2.4	0.5

Regression results indicate a significant linear trend in macroalgae cover with proximity to the inlet ($p=0.002$; $R^2=0.275$) (Fig. 2). Regression results for sponge cover indicate no significant linear trend in the data exists ($p=0.373$; $R^2=0.027$).

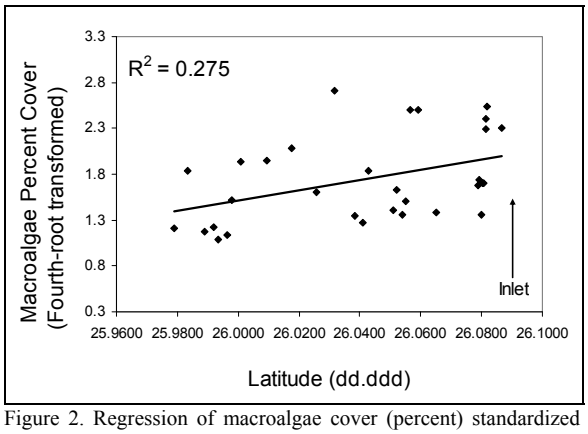

Figure 2. Regression of macroalgae cover (percent) standardized for hardbottom sampled, and fourth-root transformed, against distance from Port Everglades inlet (latitude; dd.ddd).

Water Quality Analysis

Parameters from water grab sample data near the inlet were plotted against latitude to demonstrate surface nutrient levels surrounding the inlet (Table 2; Fig. 3). Peak levels of nutrients can be observed immediately adjacent to the inlet, and dissipate with distance.

Table 2. Nutrient levels measured from grab samples taken at the surface within the inlet plume. *Denotes chlorophyll *a* sample not obtained at that location. Specific conductivity was measured at ~28°C.

Latitude	NO_2+NO_3	TKN	Total P	Chl *a*	Sp. Cond
dd	mgL^{-1}	mgL^{-1}	mgL^{-1}	μgL^{-1}	μScm^{-1}
26.08443	0.0244	0.959	0.0672	0.49	52900
26.08535	0.0305	0.535	0.0378	*	52500
26.086	0.0261	0.252	0.0366	0.81	51500
26.08712	0.165	0.648	0.039	1.06	53700
26.08783	0.0942	1.38	0.0555	*	48400
26.08867	0.0679	0.968	0.0433	*	45000
26.09117	0.0257	0.502	0.0256	*	50000
26.0920	0.061	0.461	0.0486	1.33	47100
26.0961	0.0772	1.15	0.0341	1.35	49100
26.09762	0.0736	0.692	0.0469	*	48600
26.0982	0.0266	0.812	0.0448	*	43700
26.10317	0.0296	0.746	0.0338	0.74	51200
26.10495	0.0622	1.43	0.051	*	48100

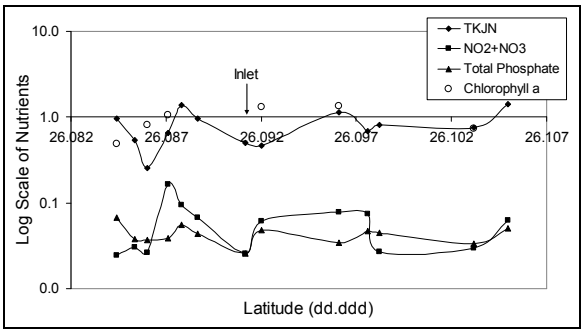

Fig. 3. Nutrient levels measured from plume water grab samples plotted against latitude across the mouth of Port Everglades inlet (log scale).

Figure 4 shows the vertical salinity gradient, or "salt wedge" in the waters immediately surrounding the inlet. Specific conductivity readings of surface water adjacent to the inlet were as low as 37,337 micro-Siemens per centimeter (μScm^{-1}), and increased as depth increased. Average oceanic conductivity ranges from 53,000-58,000 μScm^{-1} (measured between 25°C and 30°C). According to the figure, standard average ocean salinity is reached at approximately 3 m depth.

Figure 5 shows the change in surface salinity with latitude. This figure depicts the extent of the plume at the time of sampling and illustrates that specific conductivity is lowest adjacent to the inlet and increases with distance from the inlet to the north and south.

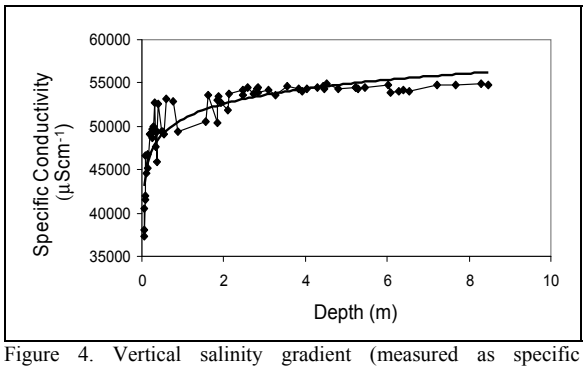

Figure 4. Vertical salinity gradient (measured as specific conductivity) across the mouth of Port Everglades inlet. Standard oceanic salinity is reached at approximately 3 m depth.

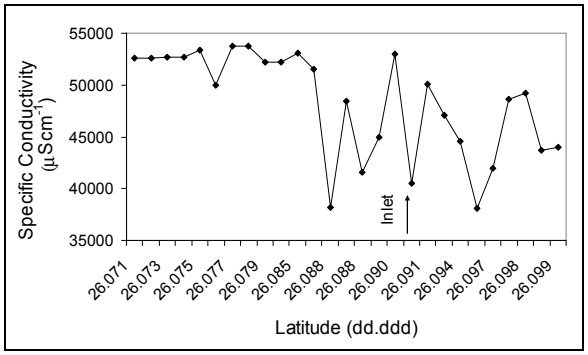

Figure 5. Change in surface salinity (measured as specific conductivity) surrounding the mouth of Port Everglades inlet with latitude. Salinity is lowest directly out from the inlet.

Regression results show that specific conductivity significantly decreases with proximity to the inlet ($p=0.0005$; $R^2=0.445$) (Fig. 6). Chlorophyll *a* was observed to increase with decreasing specific conductivity, though not significantly.

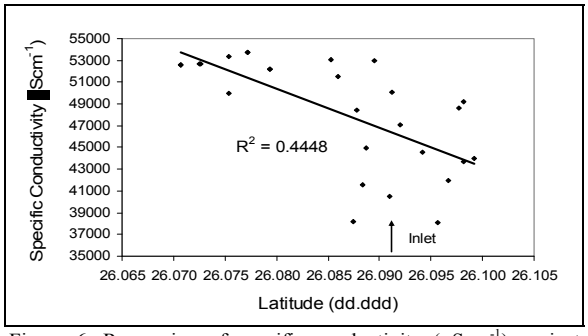

Figure 6. Regression of specific conductivity (μScm^{-1}) against distance from Port Everglades inlet (latitude).

Discussion

Because nutrients and other pollutants coming from the inlet are carried in a plume of lower-salinity water that flows as a surface "wedge" over the surrounding reef area, specific conductivity readings were used as a proxy indicator to map the approximate extent of the plume, both vertically and horizontally. The profile in Fig. 4 suggests that the plume sweeps out over the adjacent reef area at an angle influenced by the existing specific conductivity gradient (i.e. water of lower salinity will move over water of higher salinity). The combination of vertical specific conductivity profile and the general increase in surface specific conductivity with distance from the inlet (Fig. 5) suggest that benthos closer to the inlet and its discharge will be more strongly affected by the effects of lowered salinity. Lower-than-oceanic-salinity water has been proven detrimental to coral health (Linton and Warner 2003). According to Fig. 4, shallow reef areas near the inlet, especially at low tide, would be exposed to lower salinity and plume contaminants, since probe measurements do not reach normal seawater specific conductivity until approximately 3 m depth.

Abundance of inorganic nutrients in nearshore environments, particularly coral reefs, has a potentially strong influence on phytoplankton communities and trophic processes there, and has thus been recognized as a critical aspect of coastal management (Devlin et al. 2000). Dissolved nutrients in the marine environment are rapidly converted to particulate form and in turn rapidly recycled. Because of this, planktonic algae, specifically their chlorophyll *a*, have been used as a proxy indicator of nutrient abundance when monitoring water quality (Devlin et al. 1999; Linton and Warner 2003). Fig. 3 shows nutrient and chlorophyll *a* levels from grab samples highest around the mouth of the inlet, and chlorophyll *a* increased with decreasing specific conductivity (although the relationship between chlorophyll *a* and specific conductivity was not found to be significant). As specific conductivity was significantly correlated with proximity to the inlet, it appears that increased chlorophyll levels may be linked to low-salinity water, as well as increased levels of nutrients, being discharged from the inlet, although a larger sample size is desired for thorough chlorophyll *a* analysis. Though coral cover appears largely dictated by substrate variability in the nearshore area surrounding Port Everglades, corals growing near the inlet may be at risk of higher stress levels than those further from inlet influence. Heavy concentrations of phytoplankton in the water column over reefs as a result of nutrient addition may increase coral mortality due to competition for light and effected production/respiration (P/R) ratios (Marszalek 1981; Yentsch et al. 2002).

Although sponge cover was not significantly correlated with distance from the inlet, benthic macroalgae significantly increased with proximity to the channel. Water samples from each monitoring site were not obtained for nutrient measurements; however, Fig. 3 suggests that this increase in

macroalgae is due to increased nutrient availability near the inlet. Reefs experiencing nutrient addition often exhibit a shift from abundant coral to abundant macroalgae (McCrook 1999; Szmant 2002).

Although coral cover within the study areas was not significantly correlated with distance from the inlet, nearshore stony coral cover throughout Broward County south of Port Everglades inlet is naturally low, likely due to stochastic events such as hurricanes and tropical storms that frequently bury portions of the nearshore hardbottom (Prekel et al. 2008). Thus the coral-depauperate area of the nearshore benthic habitat in Broward County should be treated as a reef habitat continually under stress from natural stochastic events, as well as pollution, coastal construction and other physical impacts, and managed appropriately.

When examining the extent of the inlet plume and its influence on the surrounding benthic area, specific conductivity was found to be a reliable proxy for delineating the extent of inlet effluent plume, and abundance of benthic macroalgae and chlorophyll may be indicators of nutrient addition to the area.

It is the conclusion of these studies that Port Everglades is a source of low-salinity water, and may be discharging pollutants that can cause localized increases in phytoplankton and macroalgae abundance, all of which may be detrimental to the benthic ecology of the surrounding nearshore reef. However, lack of long-term records of variations in water quality and plume distribution make concrete determinations difficult. It is suggested here that continued benthic monitoring, as well as further water quality monitoring in proximity to Port Everglades, are needed in order to establish a more thorough analysis of the effects of inlet discharge on the surrounding nearshore reef habitat, and to make accurate predictions of changes to the habitat in the face of on-going coastal impacts.

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Coral rare earth element tracers of terrestrial exposure in nearshore corals of the Great Barrier Reef

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Abstract. Rare earth element and yttrium (REY) concentrations were measured from two nearshore *Porites* sp. corals collected from Round Top Is. and Keswick Is., at 5 km and 32 km offshore from Mackay, Queensland, on the southern Great Barrier Reef, Australia. The REY patterns from the Round Top coral differed from the Keswick coral in 5 respects: (1) greater REY concentrations; (2) preferentially enriched light rare earth elements (LREEs); (3) reduced slope across the heavy rare earth elements (HREEs); (4) more negative cerium (Ce) anomaly; and (5) lower yttrium (Y) to holmium (Ho) ratios. These patterns suggest greater terrestrial exposure and higher biological productivity at Round Top Is. Total abundance of REY in both corals increased over time between 1950 and 2002. The rate of increase at Round Top Is. was 3-fold greater than at Keswick Is., thus the innermost site was likely more influenced by weathering from rapid agricultural expansion in the adjacent Pioneer River catchment. The Y/Ho ratio decreased over time in both corals, but as the coral values were substantially higher than ambient seawater, more research is suggested to identify the cause.

Key words: Rare earth elements, yttrium, *Porites*, Great Barrier Reef

Introduction

Concentrations of rare earth elements and yttrium (REY) in coastal corals are good geochemical tracers of freshwater runoff and/or chemical weathering of continental crust because they are: (1) incorporated into coral lattices in close proportion to ambient seawater concentrations (Sholkovitz and Shen 1995); and (2) fractionated differentially in shales, river water and seawater (Elderfield et al. 1990). REY can be measured independently, or together in series, to investigate patterns that may indicate sources from local geology or land use (Lawrence et al. 2006a). Yet, the geochemical pathways from riverine transport of REY to incorporation within coral skeletons are not well studied, largely because instruments that can measure aqueous concentrations of yttrium and the monoisotopic REE with precision have only recently become available (Nozaki et al. 1997; Lawrence et al. 2006b).

River discharge is the main source of marine REY concentrations in nearshore waters (Byrne and Sholkovitz 1996). REY come from weathered topsoil and are transported in catchment waterways attached to colloidal particles within the 0.45μm fraction of the suspended sediment load (Byrne and Sholkovitz 1996). A large proportion of REY is removed in the estuarine mixing zone due to flocculation of iron-organic colloids at low salinities (Sholkovitz 1995), although large increases in REY abundances between ~5 to 10 ppt probably reflect REY release from river particles (Lawrence and Kamber 2006). Fractionation of the freshwater REY pattern also occurs within the mixing zone: light rare earth elements (LREEs) become more depleted relative to heavy rare earths (HREEs); Y fractionates relative to holmium (Ho), and a positive lanthanum (La) anomaly develops relative to its pattern in shale (Lawrence and Kamber 2006).

To identify potential terrestrial sources in marine REY, the data are typically normalized to a sediment or shale to remove the natural "saw-tooth" distribution of absolute abundances and to describe the pattern relative to a continental source (Byrne and Sholkovitz 1996). Elemental anomalies within normalized pattern, defined as departures from a smooth line predicted by extrapolation from neighboring elements (Sholkovitz 1995), can be used as "fingerprints" of biological and physical processes and/or provenance features (Akagi et al. 2004; Lawrence et al. 2006a). For example, *Porites* corals living adjacent to a soft waste dump and creek delivering runoff from an open-cut mine on Misima Island, Papua New Guinea (PNG) had positive middle rare earth element (MREE) anomalies that closely resembled those from Sepik River water (Fallon et al. 2002), suggesting that provenance information from a strong source can be preserved through the estuarine mixing zone.

In this study, REY patterns were measured from nearshore corals of the south-central Great Barrier

Reef (GBR), collected at 5 and 32 km offshore from the Pioneer River mouth and city of Mackay (Queensland, Australia). Since European settlement in 1865, there has been substantial land clearing both historically and in recent decades to support a rapidly expanding sugarcane industry (Jupiter and Marion 2008). Anomalies within REY patterns were examined to detect spatial differences in terrestrial exposure, while temporal changes in total REY load and Y/Ho ratios since the 1950s were measured to assess potential linkages to catchment land cover change.

Material and Methods

Sample preparation

Two cores (RTF, KIA) were collected from massive *Porites* colonies at reefs fringing Round Top Is. (5 km offshore) and Keswick Island (32 km offshore). Density bands from x-radiographs were used to identify years in slabs cut from each core, from which bulk samples were drilled from annual coral growth periods spanning years between 1950 and 2002. For each core, 3-5 consecutive years were analyzed from each decade. For each annual sample, approximately 5-15 µg of coral powder was diluted by 1000 with >18.2 MΩ water, HNO_3 was added to a total concentration of 2%, samples were left to digest overnight, and all solutions, including blanks, were spiked with 2 ppb of internal standard (indium (In), rhenium (Re), bismuth (Bi)).

Solution ICP-MS analyses

All samples were analyzed at the University of Queensland on a Thermo X-Series inductively-coupled mass spectrometer (ICP-MS). For instrument specifications, sensitivity and operating power, see Lawrence et al. (2006a) and Lawrence and Kamber (2006). Dilutions of USGS dolerite W-2, Mud of Queensland (MUQ; Kamber et al. 2005), and JCp-1 coral reference material (collected in 1999 from Ishigaki Island, Okinawa, Japan) were measured simultaneously with the samples for machine calibration. All REY (except promethium (Pm), which does not exist naturally in measurable concentrations; Byrne and Sholkovitz 1996), zircon (Zr) and the appropriate suite of interfering isotopes were measured. All data were corrected for drift (internal and external), oxide interferences (for europium (Eu) and heavier elements), and dilution factors.

Statistical analyses and anomaly calculations

A principal components analysis (PCA) was performed using a VARIMAX rotation to compare coral REY patterns with seawater, river water and sediment. Mean coral REY from RTF and KIA (over all years analyzed) were compared with REY data from MUQ and water samples collected from: Pioneer River mouth and estuary (Lawrence et al. 2006a); Coral Sea (Zhang and Nozaki 1996); and streams draining different regions of the Pioneer catchment (Lawrence et al. 2006a) (Fig. 1a). All REY abundances were scaled to the same value of samarium (Sm), for which anomalies are not expected, and Eu was excluded from the PCA because isobaric interference of BaO during ICP-MS analysis resulted in unreliable measurements of Eu from the Pioneer stream water samples (Lawrence et al. 2006a).

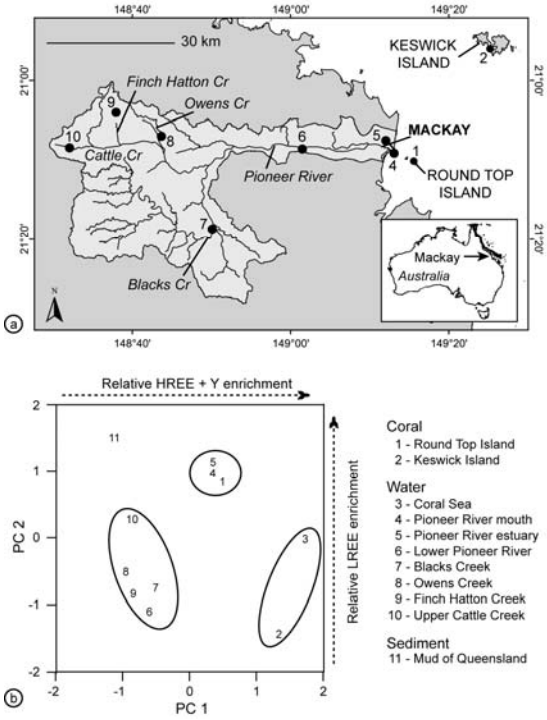

Figure 1: (a) Locations of coral core and water sample collection sites in and adjacent to the Pioneer River catchment. Inset depicts the location of Mackay within Australia. (b) Scaled REY data from coral, water and sediment samples plotted against principal components PC1 and PC2. Dashed arrows indicate directional enrichments of different groups of elements.

For assessment of deviations from the typical seawater pattern as indicators of terrestrial influence, mean coral sample REY concentrations (ppb) were normalized to those from terrestrial sediment (MUQ). To compare Mackay coral REY patterns to records from other studies, published coral REE abundances were also normalized to MUQ. The Ce anomaly was calculated using an equation from Lawrence et al. (2006b), Ce_n/Ce_n^*, where n refers to the shale-normalized abundances of Ce, praseodymium (Pr) and neodymium (Nd):

$$Ce_n^* = Pr_n * (Pr_n/Nd_n)$$

Mean differences in Ce anomalies between RTF and KIA were assessed with two-sample t-tests, after using Cochran's test to assess homoscedasticity. The ratio Lu_n/Er_n was calculated to evaluate the slope of HREE, where n refers to the shale-normalized abundances of lutetium (Lu) and erbium (Er). Because RTF and KIA had unequal variances in Lu_n/Er_n ratios, a two sample t-test with unequal variances was used to determine significance.

Multiple regressions were performed to assess changes in total REY and Y/Ho ratios from each core over time, using year and Pioneer River discharge as the independent variables. Correlations of Zr and Y/Ho ratios were checked for each core to assess detrital contamination, which would lower the Y/Ho mass ratio closer to shale values (~26) and elevate concentrations of terrestrially derived elements such as Zr (Webb and Kamber 2000). All statistical analyses were done with SYSTAT v.10.2 software.

Results

General Mackay REY patterns

When the scaled REY data were ordinated along the first two principal components, which explained 67.9% and 20.8% of the total variance respectively, the data from the most inshore coral (RTF) clustered tightly with water samples from the Pioneer River mouth and estuary (Fig. 1b). Coastal seawater, the RTF coral and terrestrial sediment (MUQ) all had high positive values along principal component 2, which are largely explained by differences in scaled LREE abundances. By contrast, the coral from further offshore (KIA) and the offshore seawater sample from the Coral Sea, had high positive values along principal component 1, which are largely explained by differences in scaled middle rare earth element (MREE) and HREE abundances. The large differences in scaled Y abundances also contribute to principle component 2, with scaled abundances of Y strongly mirroring a gradient of terrestrial influence (MUQ < Pioneer catchment streams < Pioneer River mouth < RTF < KIA < Coral Sea).

When just the coral samples were compared, RTF differed from KIA in five main respects. RTF had: (1) greater total REY abundance; (2) preferential enrichment of LREEs ; (3) flatter HREE patterns; (4) more negative Ce anomalies; and (5) smaller Y/Ho ratios (Table 1, Fig. 2). REY abundances from RTF were ~2-5 times higher than REY abundances from KIA, while LREE were preferentially enriched in RTF relative to KIA. Although shale-normalized REY from both cores were HREE enriched, RTF was less so relative to KIA, as indicated by a significantly greater (= higher slope) mean Lu_n/Er_n ratio in KIA (1.67) than in RTF (1.11) ($t = 17.41$, $df = 43$, $p < 0.001$). The mean Ce anomaly (0.502) for the RTF core was significantly lower than that (0.610) from KIA samples ($t = 6.86$, $df = 51$, $p < 0.001$). The mean Y/Ho ratio (67.3) for RTF was less than half of the mean Y/Ho (142.6) for KIA. Because there was no significant negative relationship between Zr with Y/Ho for either RTF or KIA ($r = 0.370$, $p > 0.05$ and $r = 0.045$, $p > 0.10$, respectively), these differences were not likely due to terrigenous contamination.

Temporal change

There were two major trends in the coral REY over time in both the RTF and KIA corals: (1) increased total REY; and (2) decreased Y/Ho ratios (Fig. 3a). The rate of increase in total REY abundance was approximately three times greater in RTF than in KIA, and was significantly related to both year and Pioneer River discharge: when combined they explained 49% and 46% of the total variance at Round Top and Keswick, respectively. The significance of the change in the total inshore coral REY load was more affected by river discharge at Round Top Island (partial $r^2 = 0.365$, $p < 0.002$) than at Keswick Island (partial $r^2 = 0.134$, $p < 0.025$).

The second temporal pattern in both coral records was a significant decrease of Y/Ho ratios over time, which occurred at similar rates at Round Top and Keswick islands (Fig 3b). In RTF, the trend was significantly related to year ($p < 0.001$) but not to discharge ($p < 0.059$), and together they explained 77% of the total variance. By contrast, Pioneer discharge had a more pronounced effect on the variability of Y/Ho in KIA: both year ($p < 0.002$) and discharge ($p < 0.035$) were negatively correlated with Y/Ho, but together they explained only 42% of the total variance.

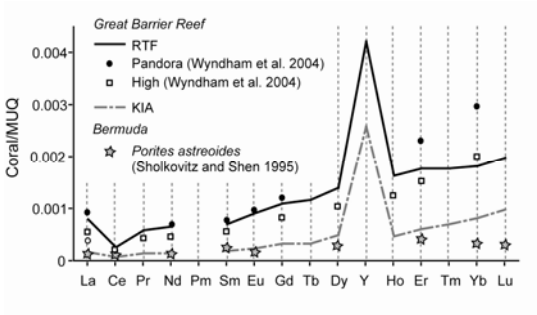

Figure 2: (a) Mean MUQ-normalized patterns in corals from Mackay (RTF: black lines; KIA: grey dashed lines) compared with other inshore GBR records (Pandora: black circles; High: white squares) and a Bermuda coral (stars).

Discussion

The presence of all of the typical marine features (La anomaly, Ce anomaly, Y/Ho fractionation, and HREE > LREE) in MUQ-normalized REY patterns from

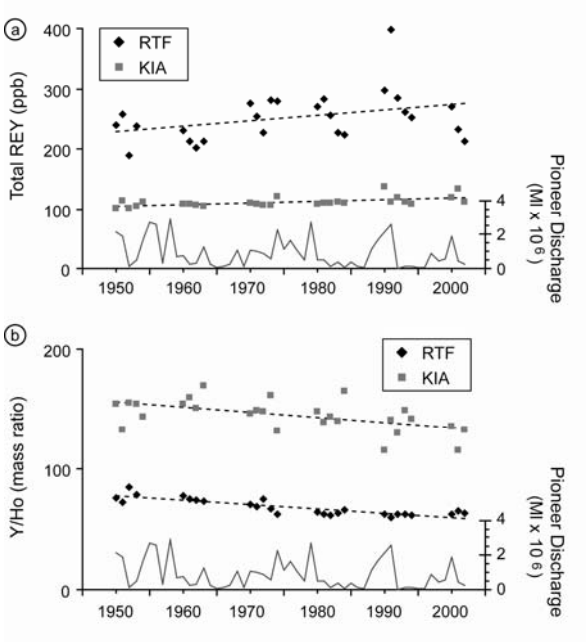

Figure 3: Temporal trends in (a) total REY concentration and (b) Y/Ho ratios between 1950 and 2002 for RTF (black diamond) and KIA (grey squares). Annual Pioneer River discharge (in megaliters) is shown with each plot.

both Mackay corals supports the assertion by previous authors that corals incorporate REY in proportion to ambient seawater concentrations (Sholkovitz and Shen 1995). However, the subtle differences in patterns between corals from Round Top and Keswick reefs likely indicate differential exposure to Pioneer River runoff and consequent differences in productivity.

The MUQ-normalized REY patterns and magnitudes from RTF were in strong agreement with other inshore GBR corals (Fig. 2) (Wyndham et al. 2004). By contrast, KIA had lower MUQ-normalized magnitudes that were similar to values from an offshore GBR reef (Wyndham et al. 2004) and values from a *Porites astreoides* collected from Bermuda (Sholkovitz and Shen 1995), suggesting reduced terrestrial influences at these sites. The underlying steady but small increase in total REY abundance over time at both sites suggests diffuse inputs of REY, possibly from greater catchment erosion coincident with agricultural expansion. Increases in REY values were most notable at Keswick in years with large floods, when plumes were more likely to reach the vicinity of the island.

LREE enrichment in the Round Top coral probably relates to greater terrestrial influence, as LREEs may be more readily mobilized by weathering than HREEs (Nesbitt et al. 1990). LREE enrichment in corals is often associated with river runoff: for example, Shioya-wan Bay (Okinawa, Japan), which receives runoff from the Taiho-o-kawa River, has flatter REY patterns (caused by LREE enrichment) and higher magnitudes of total REE in seawater and corals (including *Porites lutea*) than Sesoko-jima Island, which is distant from riverine sources (Akagi et al. 2004). When corals are sampled seasonally, LREE enrichments appear to coincide with large flood events (Wyndham et al. 2004).

The difference in the relative slopes of HREE between RTF and KIA may also be the product of terrestrial exposure. Like the water sample from the Coral Sea, the KIA coral had relative enrichments in scaled HREE abundances. While HREE enrichments are typical features of normalized oceanic seawater patterns, relative depletions in coastal waters can result from fluvial discharge, since inputs from major rivers are usually HREE depleted relative to shale (Goldstein and Jacobsen 1988). Alternatively, the flatter HREE patterns at Round Top Island may reflect differences in productivity between the two sites: Wyndham et al. (2004) noted seasonal HREE depletion in high resolution records of inshore GBR corals that they attributed to scavenging by organic (or organically coated) particles created through biological activity. Evidence of summer *Trichodesmium* blooms in the Pioneer River plume inshore from Keswick Island (Rohde et al. 2006) suggest there may be substantial differences in biological activity between the sites.

Table 1. Mean concentrations of REY in RTF, KIA, JCp-1, W-2 and MUQ. All values are in ppb, except detection limits (DL), which are in ppt (parts per trillion).

Sample	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu
RTF	26.841	19.616	5.208	22.375	5.019	1.494	7.289	1.193	8.646	138.621	2.070	6.153	0.939	6.065	0.988
DL*	0.043	0.025	0.024	0.013	0.085	0.014	0.149	0.026	0.136	0.267	0.004	0.019	0.009	0.041	0.003
KIA	5.046	4.931	1.094	4.758	1.346	0.373	2.076	0.330	2.819	82.616	0.579	2.004	0.360	2.682	0.485
DL*	0.043	0.025	0.024	0.013	0.085	0.014	0.149	0.026	0.136	0.267	0.004	0.019	0.009	0.041	0.003
JCp-1	36.333	32.886	6.485	24.433	4.850	1.214	7.499	0.970	7.076	293.684	1.807	5.562	0.776	5.446	0.789
W-2	10536	23262	3032	12937	3274	1096	3714	616	3822	20186	805	2231	328	2058	302
MUQ**	32510	71090	8460	32910	6880	1570	6360	990	5890	31850	1220	3370	510	3250	490

*DL = Detection Limit. Calculated as three times the standard deviation of background levels.
**source: Kamber et al. (2005)

Differences in Ce anomalies between the two sites may also be linked to site-specific differences in biological activity. The more negative Ce anomaly from Round Top agrees with other coral (Wyndham et al. 2004) and seawater records (Nozaki et al. 2000) showing stronger Ce anomalies in coastal regions than offshore. Wyndham et al. (2004) found strong correlations between the timing of spring-summer peaks in both Mn/Ca and Ce anomalies from inshore corals. Since Ce oxidation is probably coupled with microbially-mediated Mn oxidation, Wyndham et al. (2004) suggested that Ce oxidation increased during periods of high solar radiation and temperature, and after floods, all of which increase the abundance of oxidizing bacteria.

Explanations for Y/Ho values observed in the Mackay corals may be more complex. Due to differing surface complex stabilities, Ho is scavenged approximately twice as fast as Y within the estuarine mixing zone (Bau 1996), resulting in a superchondritic marine Y/Ho mass ratio that typically varies between ~40 and 77 (Nozaki et al. 1997; Lawrence and Kamber 2006). If corals incorporate REY in proportion to seawater concentrations, and if coastal seawater REY patterns are intermediate between riverine and offshore sites, then coastal corals would be more likely to have lower Y/Ho ratios than corals from further offshore. In relative terms, the data supported this hypothesis, but in terms of absolute Y/Ho values, the coral values may not reflect surface seawater conditions. While the Y/Ho ratio (40.5) in Pioneer River mouth seawater was at the lower end of seawater range, the mean RTF Y/Ho value (67.3) was near the upper end of the range, suggesting that: (1) the distribution coefficients for Y and Ho incorporation into coral may vary, as observed for REE measured from Bermuda corals (Sholkovitz and Shen 1995); or (2) seawater fractionation between Y and Ho may change between the river mouth and sites further offshore. The mean Y/Ho value (142.6) for KIA, while similar to that (150.8) from the reference coral JCp-1, was nearly double the upper measurements of seawater Y/Ho. Such discrepancies in coral Y/Ho values compared to their local seawater Y/Ho ratios led Webb and Kamber (2000) to suggest that, in some cases, modern microbialites (with average Y/Ho = 56.1) may actually be a better proxy for seawater REY than skeletal carbonates. These results suggest that while trends of decreasing Y/Ho may be related to increasing catchment weathering and/or exposure to freshwater, further studies are needed to better determine how the elements are incorporated into coral skeletons.

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Environmental controls on the establishment and development of algal symbiosis in corals

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Abstract: Coral reefs are under severe threat from changing climate, yet little is known about how environmental variables affect the establishment and development of coral symbiosis. Here, we use a coral larval model to test whether temperature affects the establishment and development of symbiosis using three different strains of algal symbionts (zooxanthellae) with contrasting thermal tolerance. We also compare the growth of these clades within the larval host, *Acropora intermedia*, and follow the survival of three different larval holobionts under three different temperatures (26, 30 and 34°C). Initial rates of infection were influenced by temperature in two of the three clades: in clade C9b infection rates peaked at 30°C, while in clade A6 infection rates declined with increasing temperature. Infection by clade C1 zooxanthellae was uniformly low. The thermal tolerance of the zooxanthellae did not appear to influence survival of the holobiont, however, at high temperature larvae with high densities of zooxanthellae had lower survival, suggesting zooxanthellae may be an added burden to larvae when under stress.

Keywords: coral reefs, climate change, larval ecology, reproductive biology, symbiosis

Introduction

Coral reefs are under severe threat from many sources, including rising sea-surface temperature caused by climate change (Hughes et al. 2003). A major concern is that the accelerating rate of environmental change could exceed the capacity of coral species to acclimate or adapt (Hoegh-Guldberg et al. 2007). Corals are a symbiosis between the animal host and micro-algae from the genus *Symbiodinium*. While the association is in most cases highly specific, with a given coral species associating predominantly with a single sub-clade (Knowlton and Rohwer 2003), some species associate with more than one strain and multiple strains can occur within a single colony (Rowan and Knowlton 1995, van Oppen et al. 2001). Under stress, such as high sea water temperature, the assocation can break down with a consequent loss of the algal symbionts. This process is known as coral bleaching, and can lead to the death of the coral host (Baird and Marshall 2002). Because symbiotic strains differ considerably in physiological characteristics, such as tolerance to high temperatures (Bhagooli and Hidaka 2003, Rowan 2004), a switch from a thermally susceptible to a tolerant strain is one mechanism by which corals may respond to rising seawater temperature associated with climate change (Buddemeier and Fautin 1993). This mechanism has received considerable attention in the recent literature (Baker et al. 2004), however, experimental support for it is inconclusive. For example, while there is some evidence that the identity of the symbiont can affect the physiology of the host (Little et al. 2004), it has only recently been demonstrated that heat tolerant symbionts can increase the tolerance of the host (Berkelmans and van Oppen 2006). Similarly, while a shift in the dominant clade of zooxanthellae within coral colonies has been documented following stress, such as disease (Toller et al. 2001), and transplantation (Baker 2001) there is no direct evidence to show that this change had been induced by a change in temperature nor that these shifts increase the fitness of the host (Hoegh-Guldberg et al. 2002, Douglas 2003). Furthermore, there is no evidence to show that scleractinian corals can acquire novel, and superior, symbiont clades from the

environment (Sotka and Thacker 2005). The appeal of the adaptive bleaching hypothesis is that it provides a mechanism by which corals can adjust to stress as soon as change is induced rather than over generations, as would be required via adaptation. However, changes in the composition of a species compliment of *Symbiodinium* are most likely to occur between generations at least in those species which do not inherit zooxanthellae from the parents (LaJeunesse et al. 2004). For most coral species, initial infection occurs as larvae or early juveniles, which appear, at least initially, to be much less specific in their choice of algal partner (Baird et al. 2007). Consequently, each larval recruitment event provides the opportunity for novel symbioses to be established.

The absence of zooxanthellae in oocytes of most broadcast spawning corals providing an opportunity to manipulate symbioses to explore the effects of different symbionts on the performance of the holoboint. Larvae which lack symbionts can be readily infected with many different species of symbionts under experimental conditions (van Oppen 2001, Weis et al. 2001). Consequently, it is possible to compare symbiotic and non-symbiotic individuals of the same species and to readily control the identity of both the host and the symbiont. Control over the make-up of the association is not possible in adults, because even completely bleached adults still contain residual densities of symbionts (Jones 2008) and it is yet to be established whether adults can acquire novel strains from the external environment (Hoegh-Guldberg et al. 2002).

Here, we use the coral larval model to test whether temperature effects the establishment of zooxanthella strains of contrasting thermal tolerance in larvae of the coral *Acropora intermedia*. Next we explore how the growth rate of zooxanthellae within the host is affected by temperature and finally we explore survival of three holobionts under different temperatures.

Material and Methods

We first tested the photo-physiological response of four strains of *Symbiodinium* to temperature (Fig. 1). *Symbiodinium* were isolated from four different cnidarian hosts following Yakovleva and Hidaka (2004) and identified following using internal transcribed spacer 2 (ITS2) region amplified for DGGE following Lajeunesse (2002). The host and the corresponding strains were *Seriatopora caliendrum* (C1) *Acropora intermedia* (C3), *Platygyra ryukyuensis* (C9b) and *Tridacna crocera* (A6). Isolated zooxanthellae were placed on Millipore filters and then exposed to four temperature levels, 26, 29, 32 and 34^{o}C, under a moderate light level of 110 µmol photons m^{-2} s^{-1}. A pulse amplitude modulated chlorophyll fluorometer (PAM) was used to asses the maximum quantum yield (20 min dark-adapted F_v/F_m) of zooxanthellae following Bhagooli and Hidaka (2003). Measurements were taken prior to thermal treatment (0h) and then after 6 and 12h.

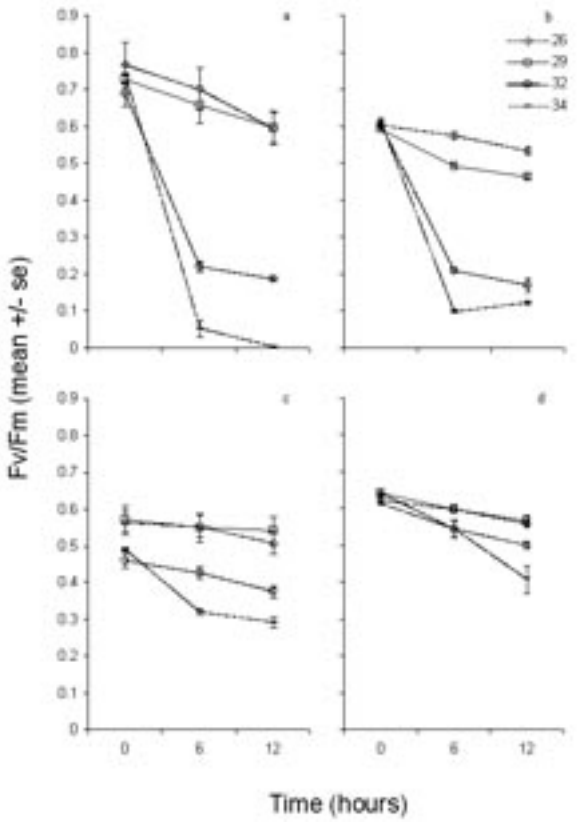

Figure 1. Thermal tolerance in four strains of zooxanthellae (a = C1, b = C3, c = A6, d = C9b).

Next we exposed larvae of *Acropora intermedia* (a species which does not contain zooxanthellae in the oocytes) collected from colonies maintained at the Churaumi Aquaria, Okinawa Japan to freshly isolated batches of three strains of zooxanthellae (all except strain C3) at densities of 4.3 x 10^6 cells per mL^{-1}at three different temperatures 26, 30 and 34C. Larvae were cultured and maintained in 0.2 FSW for three days post-spawning (Baird et al. 2006). Temperatures were maintained using temperature control units (EYELA, Thermister Tempet T-80) and larvae were kept under 110 µmol photons m^{-2} s^{-1} of PAR with a 12:12 h light: dark photoperiod. A sub-sample of 6-8 larvae was removed from each of three replicate jars after 24h and squashed under a coverslip and observed under a fluorescent microscope and the number of zooxanthellae per larva counted. In a second experiment, larvae were infected with zooxanthellae of strains C3, C9b and A6, and 50

larvae placed in 200 ml glass jars at three temperatures, 26, 30 and 32°C and larval survival was followed for 8 d. The density of zooxanthellae at the end of this time was counted to compare the growth rate of strains at each temperature.

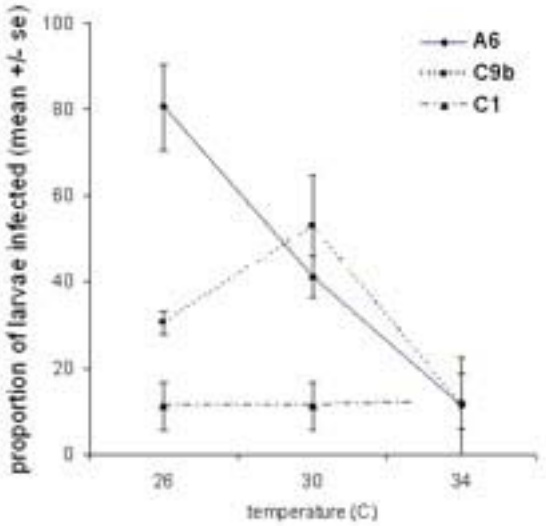

Figure 2. Percentage of *Acropora intermedia* larvae infected by zooxanthellae

Results and Discussion

The four ITS2 types of zooxanthellae clearly exhibited different functional responses to temperature (Fig. 1). Maximum quantum yield (F_v/F_m) was not affected under either 26 or 29°C treatments in any strain (Fig. 1). In strains C3 and C1, F_v/F_m dropped sharply after 6h exposure at 32°C. In contrast a significant decline in F_v/F_m did not occur until 12h at 34°C in strains C9b and A6. The zooxanthellae were consequently ranked in terms of their thermal tolerance as follows: C1 = C3 < A6 = C9b. Also of interest is the wide variation in thermal tolerance among strains of clade C, strengthening the argument that the clade of zooxanthellae is not necessarily an accurate indication of its thermal sensitivity (Toller et al. 2001). Finally, clade A zooxanthellae were both highly infective at ambient temperature, and grew rapidly within the host at temperatures up to 32°C (Fig. 3), supporting the idea that symbionts from this clade are opportunists with life history features that enable them to take advantage of ecological opportunity provided by the loss of other strains of zooxanthellae from the host following stress (Knowlton and Rohwer 2003).

Initial rates of infection were affected by temperature in two of the three symbionts (Fig. 2). In strain A6, the proportion of larvae infected decreased with temperature from 80% infection at 26°C to 10% at 34°C (Fig. 2). In strain C9b the proportion of larvae infected at 30°C was twice as high as at 26°C (Fig. 2). The proportion of larvae infected by strain C1 was uniformly low, perhaps as a result of the fact that this strain may not have a free living form and therefore be unsuitable for culture (see below).

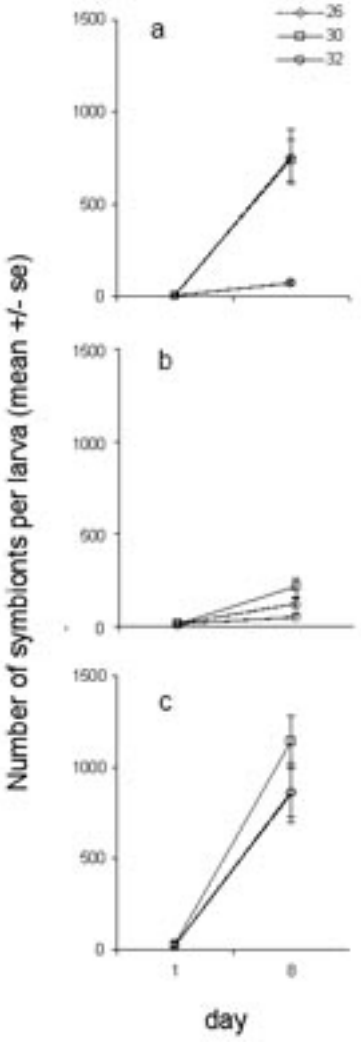

Figure 3. Change in the density of zooxanthellae strains (a = C3, b = C9b; c = A6) in *A. intermedia* larvae (n = 6).

Similarly, at 34°C the proportion of larvae infected was uniformly low (Fig. 2), suggesting none of the strains are capable of dealing with temperature 6°C above ambient. The different optimum temperature of infection in strains A6 (26°C) and C9b (30°C) suggests that certain strains may have a competitive advantage under different environments (Baird et al. 2007). However, within the range of temperatures examined these patterns of infection were not directly related to thermal tolerance of the zooxanthella strains.

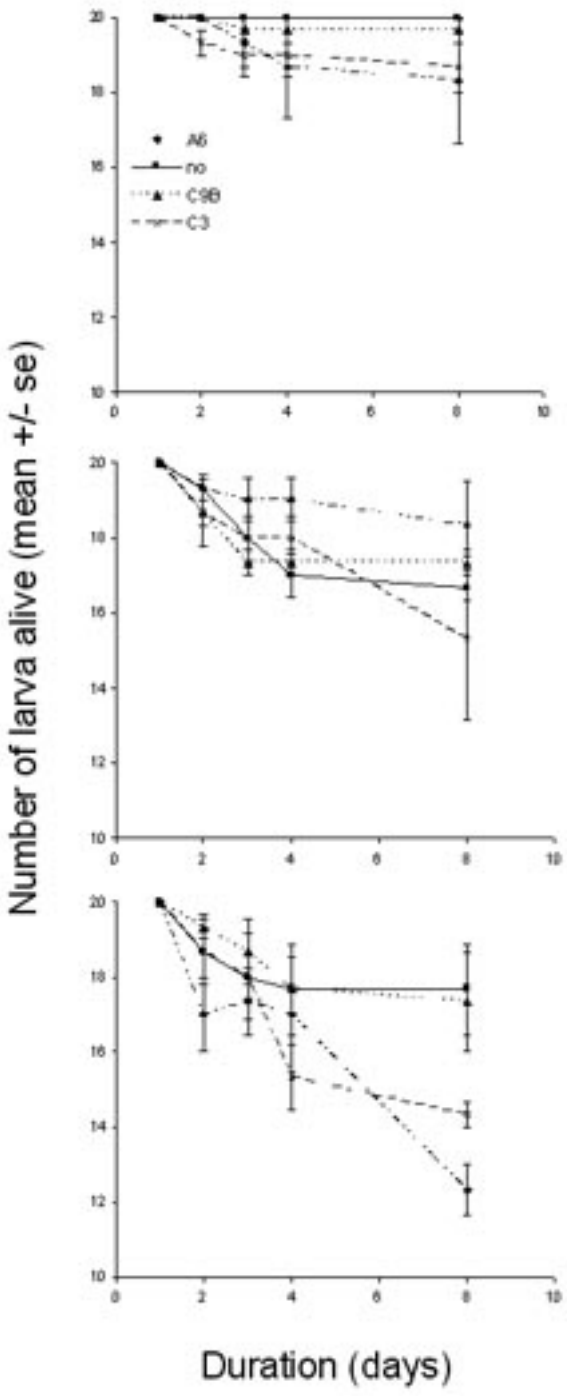

Figure 4. Survivorship of *A. intermedia* larvae infected with three strains of zooxanthellae plus non-zooxanthellate larvae at three temperatures (a = C3, b = C9b, c = A6; n = 6).

The growth rate of the zooxanthellae strains within larvae varied dramatically among strains and with temperature (Fig. 3). Strain C3 increased more quickly at higher temperature (i.e. 30 + 32 > 26) (Fig. 3). Strain C9b increase slowly at all temperatures with little difference in density among temperature treatments after 8 days (Fig. 4). Strain A6 increased rapidly at all temperatures (Fig. 3).

These data suggest there may be some trade-off in terms of thermal tolerance vs. growth rate, with strain C9b being the most thermally tolerant and also having the slowest rate of population increase in the host. Similar trade-offs between growth and tolerance have been suggested for clade D vs. clade C zooxanthellae in coral on the GBR (Berkelmans and van Oppen 2006).

There was little difference in the performance of the different holobionts at 26 or 30°C, however, at 32°C both A6 and C3 holobionts had lower survival (Fig. 4). This suggests that zooxanthellae may be an added burden to larvae when under stress (Yakovleva et al. 2009).

A number of methodological issues may have affected the results. More recent research indicates that the maximum rates of infection are not achieved until 5-7 d post spawning (Yasuda et al. 2007). Our infection experiments commenced 3 d following spawning. While a high proportion of larvae were infected in some groups, for example, 80% of larvae were infected by strain A6 at 26°C, the results may have been different if larvae were at a stage more susceptible for infection. Furthermore, the high temperature treatment was probably too extreme. Very few larvae were infected by any strains at 34°C which is nearly 6°C above ambient at this time of year in Okinawa. Future work should examine infection using more treatments over a narrower range to more completely describe the functional response of larval infection to temperature. Also, other differences between some of the zooxanthellar strains could potentially affect rates of infection. In particular, strain A6 is not an intra-cellular symbiont, rather it is found in the haemal sinuses of the clam mantle (Farmer et al. 2001). Also, strain C1, isolated from *Seriatopora caliendrum*, is transmitted vertically i.e. directly into the oocytes of offspring and therefore may not exist outside the host. In contrast, strains C3 and C9b are both inherited horizontally i.e. they must be acquired anew in each generation, and therefore must have a free living stage in their life history. Finally, to effectively explore the effect of functionally different strains on the host performance it would have been ideal to wait until zooxanthella densities had stabilized within the host. This may take 4-6 weeks in coral larvae (Baird et al. 2006) which have the potential to host 1000s, to tens of thousands of zooxanthellae per larvae (Edmunds et al. 2005).

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Does the coral host protect its algal symbionts from heat and light stresses?

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Abstract. This study investigated the potential protective role of the host by exposing coral larvae infected with two distinct clades of *Symbiodinium* (A and D) in two conditions, cultured and *in hospite*, to three combinations of heat and light levels (control: 24 °C + 40 μmol photons m^{-2} s^{-1}; high temperature and low light (HT+LL): 31 °C + 40 μmol photons m^{-2} s^{-1}; high temperature and high light (HT+HL): 31 °C + 390 μmol photons m^{-2} s^{-1}). HT+HL treatments decreased maximum quantum yield of photosystem II, F_v/F_m, in both cultured and *in hospite* zooxanthellae, however, the effect was more pronounced in cultured samples. HT+LL led to a decrease in maximum relative photosynthetic electron transport rate ($rETR_{max}$) only in cultured algae, while high light reduced $rETR_{max}$ in both *Symbiodinium* growth conditions, with $rETR_{max}$ approaching zero in cultured *Symbiodinium*. When *Symbiodinium* cells *in hospite* were exposed to HT+HL treatments, non-photochemical quenching increased; however for cultured *Symbiodinium* it declined drastically indicating that this particular form of photoprotection was depleted. These results suggest that *in hospite Symbiodinium* sustain less photo-physiological damage from HT+HL treatments in comparison to their respective free-living counterparts suggesting that the coral host provides some degree of protection to *Symbiodinium* from thermal and light stresses.

Key words: Coral host, photo-physiology, stress, *Symbiodinium*.

Introduction

Symbiosis between corals and zooxanthellae (*Symbiodinium* spp.) is dependent upon the mutual exchange of nutrients, with the coral receiving photosynthetically fixed carbon (Muscatine 1990) in exchange for nitrogen and phosphorus from the host. The association is beneficial to the coral host because it gains an additional metabolic capability, which has been hypothesized as the reason why corals have been known to expand into a previously inhospitable environment, the oligotrophic waters of the world's tropical oceans (Douglas 1994). However, the endosymbiotic zooxanthellae may also have gained a similar benefit from their host. Shallow tropical waters are characterized as being a highly variable light and temperature habitat. The host could provide some photoprotection for the symbionts, whereas these conditions would be most challenging for the free-living equivalents of zooxanthellae. It is therefore possible that the host could provide photoprotection, as well as nutritional support for its symbiotic partner.

Non-photochemical quenching (NPQ) is a fluorometric response mostly driven by a decline in Fm' (light-adapted maximum fluorescence). The closure of PSII reaction centres is one cause of reduced *Fm'*. When a photosystem experiences high light, electron transport increases as does production of protons, leading to a pH gradient across the thylakoid membrane. This gradient in turn triggers the epoxidation of xanthophyll pigments to dissipate excess photon energy in the form of heat; this is known as NPQ.

Coral bleaching occurs when zooxanthellae are released or their pigments are degraded. This disassociation of the host and the algal symbiont has been linked in most cases to the thermally-induced dysfunction of the *Symbiodinium* photosynthetic machinery, in particular at the dark reactions (Jones et al. 1998) and/or PSII light reactions (Warner et al. 1996, Lesser & Farell 2004, Hill et al. 2004, Bhagooli & Hidaka 2006, Hill & Ralph 2006). Photosystem II (PSII) and the photosynthetic electron transport rate of *Symbiodinium* are, directly and/or indirectly via

dark reactions, altered under thermal and/or light stresses (Bhagooli & Yakovleva 2004). A range of genetically distinct clades of symbionts has been found in association with corals, clams and other reef invertebrates. Differential coral bleaching due to environmental stress in adult cnidarians harboring different clades of *Symbiodinium* has also been documented (Rowan 2004, Berkelmans & van Oppen 2006).

The present study used two strains of *Symbiodinium* that were infected into aposymbiotic larvae and exposed to thermal and/or light stresses to investigate the potential role of the coral larval host in protecting their symbionts' photo-physiology.

Material and Methods

Four colonies of the broadcast spawning coral *Favia pallida* were collected from the reef crest at Little Pioneer Bay on Orpheus Island (18°46'S, 146°15'E) in the Central Great Barrier Reef (GBR), Australia, on the night prior to spawning (19th November 2005) and transported into flow through aquaria at the Orpheus Island Research Station. After spawning, larvae were cultured following the methods of Babcock et al. (2003) and maintained in 1-L glass jars in 0.02 µm filtered seawater (FSW) at initial densities of approximately 500 larvae per litre under cool-white fluorescent light tubes at a an irradiance of 40-60 µmol photons m^{-2} s^{-1} with a 12:12 h light: dark photo-period at temperature between 25 – 26 °C. FSW was changed weekly.

One group of larvae was infected with cultured (f2 media) free-living zooxanthellae obtained from the Australian Commonwealth Scientific and Industrial Research Organization (CSIRO) laboratories. These zooxanthellae were originally isolated from the clam, *Hippopus hippopus*, and have recently been genotyped as clade A (K. Ulstrup pers comm.). The second clade of zooxanthellae was isolated from *Acropora millepora* colonies from Geoffrey Bay on Magnetic Island (19° 09'S, 146°51'E) in the Central GBR. This population contains almost exclusively clade D zooxanthellae (Little et al. 2004). Zooxanthellae were isolated from adult tissue using a Water-Pik® containing FSW. Coral blastate was first filtered through a 350 µm nylon mesh to remove the mucus, homogenized and then centrifuged at 2,000 g for 5 min. The resulting algal pellet was re-suspended in FSW and centrifuged and this process was repeated twice to remove all animal tissue. Finally, zooxanthellae were re-suspended and added to the larval cultures at very high densities (600-800 cells). Zooxanthellate isolates were introduced to larval cultures within 30 min of preparation.

Three larvae infected with either clade A or D *Symbiodinium*, were exposed to three treatments: 24°C under 40 µmol photons m^{-2} s^{-1} light level (control LT+LL), 31 °C and 40 µmol photons m^{-2} s^{-1} (HT+LL), and 31°C and 390 µmol photons m^{-2} s^{-1} (HT+HL) for 8 h. Single larva in a transparent tube (cc. 2 ml) filled with 2 ml FSW was exposed at each combination of temperature and light treatment. Photosynthetic parameters describing the condition of the *Symbiodinium* were assessed using a PAM. After each trial, the fluorometer was used to measure rapid light curves (RLCs) followed by a 10 min dark-adapted maximum quantum yield of PSII, (F_v/F_m) measurement at the end of the 8 h exposure period. To measure the photo-physiological responses of the *in hospite* symbionts using the Microscopy-PAM, the larvae were transferred to glass slides with cover slip gently placed on top of them.

Fluorescence measurements of single *Symbiodinium* cells were performed using the Microscopy-PAM (Walz, Effeltrich, Germany) (Ralph et al. 2005). Maximum and effective quantum yield of PSII were determined according to the following expressions $(F_m-F_o)/F_m = F_v/F_m$ and $(F_m'-F)/F_m'$ or Φ_{PSII} where F_m is the dark-adapted maximum fluorescence, F_o is dark-adapted minimum fluorescence and F_m' is the light-adapted maximum fluorescence and F is the fluorescence prior to a saturating pulse (Genty et al. 1989). Electron transport rate (ETR) and non-photochemical quenching (NPQ) were estimated from the RLCs. The relative electron transport rate (rETR) was calculated as rETR = Φ_{PSII} X PAR. The rETR measurements were obtained by applying a series of saturating pulses under increasing actinic irradiance (0, 15, 50, 90, 150, 200, 250, 300, 350 µmol photons m^{-2} s^{-1}) at 10 s intervals yielding a RLC. The non-photochemical quenching (NPQ) derived from the expression $(F_m-F_m')/F_m'$ was determined as the highest NPQ value on the RLC. A double exponential decay function (Platt et al. 1980) was used to fit curves to the RLCs and quantitatively compare descriptive parameters such as α (initial slope of the RLC before the onset of saturation), E_k (minimum saturating irradiance) and $rETR_{max}$ (maximum relative electron transport rate) (Ralph et al. 2002).

Platt et al. (1980) equation:

$$P = P_s(1-e^{-(\alpha Ed/Ps)})e^{-(\beta Ed/Ps)}$$

Where P_s is the maximum potential rETR, α is the initial slope of the RLC, E_d is the downwelling irradiance (400-700 nm) and β is the slope of the RLC beyond the onset of photoinhibition. In the absence of photoinhibition ($\beta = 0$), the function can be simplified as

$$P = P_m(1 - e^{-(\alpha Ed/Pm)})$$

Where P_m is the photosynthetic capacity at saturating irradiance.
$rETR_m$ and E_k were estimated by the following equations:

$$rETR_m = P_s[\alpha / (\alpha + \beta)]/[\beta / (\alpha + \beta)]^{\beta/\alpha}$$

and

$$E_k = rETR_m/\alpha$$

High temperature with either low or high light treatment data were normalized to control values (24 °C + 40 µmol photons m^{-2} s^{-1}) from respective treatments to allow reliable comparison to be made between *in hospite* and cultured *Symbiodinium*. A 3-way ANOVA was performed to analyse the interactions among clade, growth condition and light. Tukey pairwise comparison of means was used to determine significant differences between treatments, $P < 0.05$.

Results

Symbiodinium growth condition (culture vs *in hospite*) but not clade (A vs D) had a significant effect on all the chlorophyll *a* fluorescence parameters measured (Table 1). Light alone significantly affected F_v/F_m, $rETR_{max}$, NPQ and α. Significant interactions of clade and light were observed for F_v/F_m only. An interaction between growth condition and light was observed for $rETR_m$, NPQ and E_k.

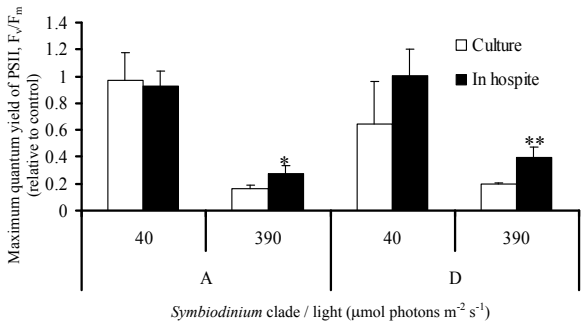

Figure 1: Chlorophyll fluorescence ratio, (F_v/F_m), of cultured (empty bars) and *in hospit*e (filled bars) clade A and clade D *Symbiodinium* exposed to low light (31°°C+ 40 µmol photons m^{-2} s^{-1}) and high light (31°C+390 µmol photons m^{-2} s^{-1}) for 8 h expressed as relative to their respective control (24°C + 40 µmol photons m^{-2} s^{-1}). Data represent means ± SD, n=3. Asterisks represent significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$ for *, ** and ***, respectively.

High temperature plus high light treatments (HT + HL) significantly affected all chlorophyll fluorescence parameters measured in both exposure conditions except for E_k for *in hospite Symbiodinium* (Table 2). Under HT+HL conditions F_v/F_m (Fig. 1), $rETR_{max}$ (Fig. 2A) and α (Fig. 2B) declined significantly to less than 20, 9 and 25% of control values in culture and 40, 60 and 64% of control values for *in hospite Symbiodinium*, irrespective of clade.

Table 1. 3-way ANOVA analyses of (a) F_v/F_m and rapid light curve derived parameters (b) $rETR_{max}$, (c) relative NPQ, (d) relative α and (e) relative E_k. Clade = *Symbiodinium* A and D; growth condition (GC) = culture and *in hospite*; Light = 40 and 390 µmol photons m^{-2} s^{-1}. Asterisks represent significant difference $P < 0.05$.

Source of variation	SS	df	MS	F	*P*
a) F_v/F_m					
Clade	0.002	1	0.002	0.175	0.681
GC	0.197	1	0.197	19.608	0.000*
Light	1.806	1	1.806	180.003	0.000*
CladeX GC	0.037	1	0.037	3.720	0.072
Clade X Light	0.65	1	0.65	6.512	0.021
GC X Light	0.035	1	0.035	3.526	0.079
Clade X GC X Light	0.01	1	0.01	1.005	0.331
b) $rETR_{max}$					
Clade	1.667E-05	1	1.667E-05	0.065	0.803
GC	0.073	1	0.073	281.032	0.000*
Light	0.120	1	0.120	466.129	0.000*
Clade X GC	0.001	1	0.001	2.323	0.147
Clade X Light	1.667E-05	1	1.667E-05	0.065	0.803
GC X Light	0.008	1	0.008	31.226	0.000*
Clade X GC X Light	0.000	1	0.000	0.000	1.000
c) Rel. NPQ					
Clade	0.001	1	0.001	0.771	0.393
GC	0.831	1	0.831	552.738	0.000*
Light	0.777	1	0.777	516.933	0.000*
Clade X GC	0.000	1	0.000	0.226	0.641
Clade X Light	1.322E-05	1	1.322E-05	0.009	0.926
GC X Light	3.226	1	3.226	2144.813	0.000*
Clade X GC X Light	0.001	1	0.001	0.399	0.537
d) Rel. α					
Clade	0.000	1	0.000	0.189	0.669
GC	0.025	1	0.025	13.853	0.002*
Light	0.116	1	0.116	65.161	0.000*
Clade X GC	0.005	1	0.005	2.544	0.130
Clade X Light	3.75E-05	1	3.75E-05	0.021	0.887
GC X Light	0.004	1	0.004	1.965	0.180
Clade X GC X Light	3.75E-05	1	3.75E-05	0.021	0.887
e) **Rel E_k**					
Clade	1.76E-05	1	1.76E-05	0.001	0.975
GC	0.398	1	0.398	22.611	0.000*
Light	0.075	1	0.075	4.260	0.056
Clade X GC	0.02	1	0.02	1.131	0.303
Clade X Light	0.001	1	0.001	0.075	0.787
GC X Light	0.126	1	0.126	7.137	0.017*
Clade X GC X Light	0.002	1	0.002	0.125	0.728

Minimum saturating irradiance (E_k) in both *Symbiodinium* clades declined to less than 50% of control in culture condition only (Fig. 2C). NPQ for both *Symbiodinium* clades exhibited opposite trends (Fig. 2D). In culture, NPQ decreased to less than 20% of control, while for *in hospite* it increased by more than 2-fold relative to control values. High temperature and low light treatments did not significantly affect F_v/F_m (Fig. 1), α (Fig. 2B) and E_k (Fig. 2C) both in culture and *in hospite* states but significantly altered $rETR_{max}$ (Fig. 2A) and NPQ (Fig. 2D) in culture growth form only (Table 2). Exposure to HT+LL significantly decreased $rETR_{max}$ to less than 75% of control values but increased NPQ by more than 2-fold in culture and *in hospite* conditions in both *Symbiodinium* clades.

Table 2 Chlorophyll fluorescence parameters – F_v/F_m (dark-acclimated), ETR_{max}, α, E_k (from curve fitting of RLC), and NPQ (from RLC). Values are means ± SD. One factor analysis of variance and Tukey's pairwise comparison of means for $P < 0.05$; superscript letters represent similar means. Asterisks represent significant difference $P < 0.05$. For treatments see text.

Parameter	Treatment	Clade A		Clade D	
		Culture	*In hospite*	Culture	*In hospite*
F_v/F_m	Control	0.53±0.05^a	0.53±0.09^a	0.53±0.04^a	0.52±0.06^a
	HT+LL	0.51±0.06^a	0.48±0.07^a	0.34±0.17^a	0.514±0.04^a
	HT+HL	0.09±0.02^b	0.16±0.07^b	0.10±0.1^b	0.21±0.05^b
	P-value	0.000*	0.000*	0.004*	0.001*
ETR_{max}	Control	43.57±2.81^a	67.43±9.92^a	42.23±0.60^a	68.47±3.46^a
	HT+LL	30.50±5.57^b	79.53±10.03^a	31.37±4.65^b	75.93±3.56^a
	HT+HL	3.75±1.37^c	40.23±8.40^b	3.96±0.86^c	36.00±1.90^b
	P-value	0.000*	0.008*	0.000*	0.000*
α	Control	0.51±0.02^a	0.40±0.14^a	0.50±0.04^a	0.68±0.04^a
	HT+LL	0.46±0.12^a	0.48±0.06^a	0.50±0.09^a	0.72±0.02^a
	HT+HL	0.10±0.05^b	0.22±0.01^b	0.12±0.04^b	0.32±0.06^b
	P-value	0.001*	0.033*	0.000*	0.000*
E_k	Control	86.01±5.36^a	182.90±53.22	85.29±5.12^a	100.47±9.07
	HT+LL	68.80±16.35^{ab}	168.07±26.03	63.61±6.17^a	105.58±7.54
	HT+HL	41.76±12.42^b	211.47±85.74	34.53±12.96^b	115.79±18.23
	P-value	0.013*	0.832	0.002*	0.378
NPQ	Control	0.23±0.02^a	0.24±0.02^a	0.24±0.02^a	0.23±0.00^a
	HT+LL	0.54±0.02^b	0.23±0.02^a	0.55±0.00^b	0.24±0.02^a
	HT+HL	0.04±0.01^c	0.56±0.01^b	0.05±0.01^c	0.55±0.01^b
	P-value	0.000*	0.000*	0.000*	0.000*

Discussion

Here we show that the photo-physiology of zooxanthellae *in hospite* was better able to cope with heat and light stress than zooxanthellae of the same clade in culture suggesting that the coral host provides important protection to the photosynthetic apparatus of algae.

Maximum quantum yield of PSII (F_v/F_m) and rapid light curves derived parameters (α, $rETR_{max}$ and E_k) were affected by temperature and/or light stresses with *Symbiodinium* in culture being more sensitive than those *in hospite*, irrespective of clade. Maximum quantum yield of PSII (F_v/F_m) and efficiency of photosynthesis (α) both exhibited similar declines under thermal and light stresses (Fig. 2). A high temperature of 31 °C for 8 h when combined with low light did not affect F_v/F_m or α, however when combined with high light conditions did significantly reduce F_v/F_m and α of *Symbiodinium* in both culture and *in hospite* conditions, irrespective of clade. Iglesias-Prieto et al. (1992), also found temperatures above 30 °C impaired photosynthesis in cultured *Symbiodinium*, and Jones et al. (1998), Warner et al. (1996) and Hill & Ralph (2006) reported decreased F_v/F_m and α values in *in hospite Symbiodinium* when adult corals were exposed to 32 °°C under moderate (350 µmol photons m^{-2} s^{-1} or above) light levels, that is bleaching/stress conditions.

The capacity of photosynthesis ($rETR_{max}$) significantly decreased under HT+LL conditions only in cultured *Symbiodinium*, while HT+HL induced far greater photosynthetic impacts. $rETR_{max}$ of i*n hospite Symbiodinium* declined when exposed to HT+HL. Decreases in $rETR_m$ due to elevated temperature exposure are possibly related to reduced Rubisco activity (eg Lesser & Farell 2004). Another possible explanation for the latter results might be the relative light climate and optical filtering offered by the coral larval host.

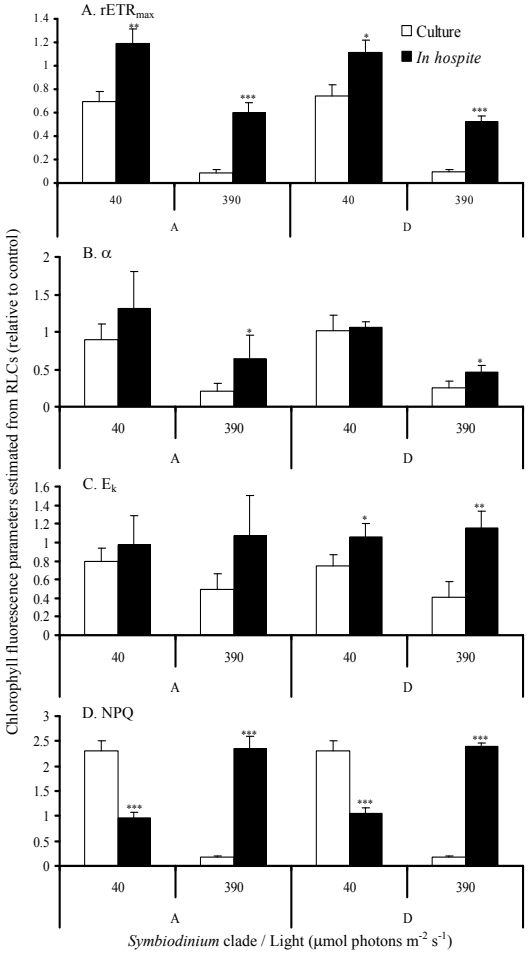

Figure 2: Derived chlorophyll fluorescence parameters ETR_{max} (A), α (B) and E_k (C) from curve fitting of RLC, and NPQ_m (D), the highest value from RLC, of cultured (empty bars) and *in hospit*e (filled bars) clade A and clade D *Symbiodinium* exposed to low light (31ºC+ 40 µmol photons m^{-2} s^{-1}) and high light (31ºC+390 µmol photons m^{-2} s^{-1}) for 8 h expressed as relative to their respective control (24ºC + 40 µmol photons m^{-2} s^{-1}). Data represent means ± SD, n=3. Asterisks represent significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$ for *, ** and ***, respectively.

Symbiodinium cells *in hospite* were less affected than those cells maintained as free-living cultures, which tends to reflect different temperature stress thresholds for cultured (30 °C) (Iglesias-Prieto et al. 1992) and *in hospite* (32 °C) *Symbiodinium* of some corals. At HT+LL, $rETR_{max}$ decreased while NPQ increased, without any change in F_v/F_m, α and E_k, in cultured growth form only. This implies that the photosynthetic apparatus of cultured *Symbiodinium* under HT+LL was under significant stress. *In hospite Symbiodinium* did not exhibit any significant change in $rETR_m$ and NPQ under HT+LL conditions (Fig. 2). Under HT+HL conditions, the F_v/F_m, $rETR_{max}$, α, E_k

and NPQ decreased drastically in free-living *Symbiodinium*, indicating collapse of photosynthesis. *In hospite Symbiodinium* exhibited a significantly reduced decline in F_v/F_m, $rETR_{max}$ and α, increases in NPQ and no change in E_k suggesting reduced levels of photosynthetic stress. These findings are indicative of the potential role of the host as a buffer in protecting its photosynthetic symbionts. Whether it is just the optical filtering by the host or host related changes in pH, dissolved inorganic carbon (DIC) supply or access that provide photoprotection for the symbiont needs further investigation. Larvae usually possess photoprotective pigments called green fluorescent proteins (Bhagooli pers. obs.), as do adult corals (see review in Baird et al. 2009). Thus, it is reasonable to expect that the larval host can act as thermal and/or light protector to the algae under certain exposures, beyond which both the host and the algae would be stressed.

The findings that clade did not have an effect on the *Symbiodinium* responses to thermal and/or light stresses are not consistent with those of Rowan (2004) and Berkelmans & van Oppen (2006), which clearly showed that clade D *Symbiodinium* is more thermally tolerant than clade C. In the present study, clades A and D *Symbiodinium* responded similarly to thermal and/or light stresses. Rowan (2004) and Berkelmans & van Oppen (2006) found that a significant decline in F_v/F_m was observed after exposing adult corals harboring clade D to 32 °C for several days. Cultured and *in hospite* (within larvae) *Symbiodinium* cells showed a decrease in F_v/F_m after 8 h exposure at 31 °C and 390 µmol photons m^{-2} s^{-1} implying that clade D *Symbiodinium* is more thermally sensitive when held in cultured condition and when inside coral larvae compared to reported thermal responses of clade D in adult corals. It is noteworthy that Tchernov et al. (2004) has documented intra-cladal variation in F_v/F_m responses to thermal stress.

This study showed that *Symbiodinium* cells *in hospite* are more photosynthetically robust than when free-living and suggests a role of the host in protecting its symbionts and providing access to an environment that would otherwise be uninhabitable. Further studies are needed to explore light attenuation by host tissue in order to compare the amount of light that symbionts are exposed to when they are *in hospite* and in culture conditions. Moreover, investigations related to changes in pH, dissolved inorganic carbon supply or access to it might provide further mechanistic explanation for the host role in protecting its symbionts from environmental stresses.

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Measuring *Symbiodinium* sp. gene expression patterns with quantitative real-time PCR

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Abstract Quantitative real-time PCR is a popular method for measuring gene expression in many biological organisms. The dinoflagellate *Symbiodinium* spp. lives in eukaryotic symbioses with reef-building corals and other marine invertebrates and protists. The use of quantitative real-time PCR to measure gene expression levels of the dinoflagellate symbiont has been limited by the lack of validated normalisation genes and the inability to purify mRNA from the alga without significant host contamination. Normalisation genes to correct for errors inherent to this technique are essential and as yet no validated normalisation genes have been identified for use in *Symbiodinium* gene expression studies. Three commonly used normalisation genes, β-actin, proliferating cell nuclear antigen (PCNA) and 18S rRNA, are tested for use as internal standards in light manipulation experiments of the major light harvesting protein complexes (chlorophyll *a* chlorophyll c_2 peridinin protein complexes - acpPC). Using the algorithm software program geNorm, β-actin and PCNA were found to be more stably expressed than the more highly expressed 18S rRNA genes, and result in similar gene expression profiles when used to normalise gene expression data for three different acpPC genes.

Key words: qRT-PCR, normalisation genes, *Symbiodinium* sp, light harvesting protein complexes

Introduction

The use of reverse transcription in conjunction with quantitative real-time polymerase chain reaction (qRT-PCR) is now a fundamental technique for measuring transcript levels of genes in a wide variety of organisms. The most commonly used approach involves the quantitation of transcript levels in relation to the level of an experimentally stable internal reference or normalisation gene. Alternatively absolute copy numbers of the transcript of interest can be calculated from a standard curve. qRT-PCR, while relatively straight forward to perform, has technical problems. These include RNA variability, variability in extraction protocols and different reverse transcription and PCR efficiencies (Bustin and Nolan 2004), while analysis of results is complicated by the method used for normalisation (Dheda et al. 2004). Appropriate selection of normalisation genes is important in controlling for these inherent problems, but the selection of an unstable gene may statistically influence results by either masking the detection of small changes or providing an incorrect result (Dheda et al. 2005). While growing numbers of studies test the potential of normalisation genes for use with organisms such as plants (Olbrich et al. 2008), Chromalveolata (Siaut et al. 2007) and Cnidarians (Rodriguez-Lanetty et al. 2008), the vast majority continue to use plasmid DNA or cell number (Demir et al. 2008) to quantify absolute transcript levels (Table 1). Use of qRT-PCR to measure gene expression where both partners are symbiotic eukaryotes is significantly more complicated than studies with single organisms or symbioses between eukaryote and prokaryote partners. The greatest challenge with symbioses between two eukaryote partners involves differentiating RNA contribution of the host organism from the symbiont and determining variability in the RNA contribution between samples.

In dinoflagellate symbioses studies qRT-PCR is primarily used to determine or compare the presence of different algal populations (example Loram et al. 2007) within scleractinian corals. In these studies use of normalisation genes or absolute transcript copy numbers is not necessary. Future approaches to eukaryotic symbioses studies are expected to incorporate exogenous RNA spikes. This enables quantification of symbiont contribution to RNA extracts, as was elegantly demonstrated for anthozoans harbouring endosymbiotic dinoflagellates (Mayfield et al. 2008). While utilisation of qRT-PCR to measure gene expression of coral or their single-celled phototrophic dinoflagellate of the genus *Symbiodinium* is still in the initial stages, it will become an important tool for elucidating the effects of increasing anthropogenic and environmental stresses on this important symbiotic relationship.

Here validation of three commonly used normalisation genes, 18S rRNA, β-actin and proliferating cell nuclear antigen (PCNA) are tested for stability under varying light conditions for future gene expression experiments with *Symbiodinium* sp. light harvesting chlorophyll *a* chlorophyll c_2 peridinin protein complex (acpPC) genes.

Table 1: Common normalisation methods used in plant, algae, symbiosis and bacteria.

Normalisation Standard	Organism
β-actin	Cnidaria (example Deboer et al. 2007) Dinoflagellate (Kobiyama et al. 2005) Plant (Jurca et al. 2008)
18S rRNA	Plant (Giorio et al. 2007)
GAPDH	Plant (Jurca et al. 2008)
Plasmid DNA or cell numbers	Dinoflagellates (example Demir et al. 2008) Raphidophytes (example Handy et al. 2008) Pelagophytes and haptophytes (John et al. 2007) Cyanobacteria (John et al. 2007)

Material and Methods

Symbiodinium *samples*

Coral branches from three colonies of *Acropora aspera* hosting *Symbiodinium* (clade C3) were collected from Heron Island (Great Barrier Reef (23°33'S, 151°54'E) in June 2005 and placed in four flow through aquaria. Light levels in two aquaria were reduced by shading (average daily light dosage 0.7 mol quanta m^{-2} d^{-1}), while the two remaining tanks were left exposed (average daily light dosage of 17.4 mol quanta m^{-2} d^{-1}). Over the course of the experiment the maximum irradiance recorded in the shaded tanks ranged between 27.3 and 98.9 µmol quanta m^{-2} s^{-1}, while the exposed tanks ranged from 573 to 1540 µmol quanta m^{-2} s^{-1}. One sample from each colony was collected from each tank at 1300 h on days 1, 3, 5, 7 and 9 of the experiment for RNA extraction.

RNA isolation

Symbiodinium were removed from the coral skeleton using an oral irrigator (WaterPik™) into 70 ml of 0.45 µm filtered sea water (FSW). The resulting homogenate was centrifuged for 2 min at 5000 g, the supernatant discarded and pellet resuspended and washed in 5 ml of FSW. This protocol was repeated a second time before the pellet was transferred to a clean tube and frozen in liquid nitrogen and stored at -80°C. Algal pellets were ground under liquid nitrogen and total RNA isolated using a RNeasy Plant Mini kit (Qiagen, Valencia USA). RNA was qualitatively assessed by a 0.9% formaldehyde agarose gel and quantified using a NanoDrop-1000 (NanoDrop Technologies, Wilmington USA).

qRT- PCR analysis

Reverse transcription and genomic DNA elimination was performed with QuantiTect Reverse Transcription kit (Qiagen, Valencia USA) in a 20 µl reaction using 500 ng of total RNA as template and a RT primer mix consisting of random primers and oligo-dT. Template dilution series were prepared to optimize quantification accuracy. After 4 fold dilution with ddH_2O, 3 µl of diluted template was analysed using the Rotor-Gene™ 6000 (Corbett Life Science, Australia). The PCR was performed with 7.5 µl of Platinium® SYBR® Green qPCR SuperMix-UDG (Invitrogen Corp, Carlsbad, USA) and gene specific primers (200 nM) for either β-actin, PCNA, 18S rRNA or one of three acpPC genes. The protocol was 95°C for 2 min, followed by 45 cycles of 15 s at 95°C and 30 s at 60°C with the temperature increasing 1°C every 5 s from 66°C to 95°C in a final melt stage. Each GeneDisc™-100 (Corbett Life Sciences, Australia) included reactions for one normalisation and one *Symbiodinium* acpPC gene, with samples run in triplicate and non-template controls in duplicate. Standard curves for each normalisation and *Symbiodinium* acpPC gene were generated on every run with five duplicates using a single template sample. An additional PCR efficiency check using four different RNA templates was conducted to ensure inter-disc comparability.

Primers for β-actin, PCNA and 18S rRNA were designed using combinations of MacVector Inc (USA) and DNASTAR Lasergene Primer Select (USA) based on alignments of multiple *Symbiodinium* cDNA sequences obtained from the NCBI GenBank database (www.ncbi.nlm.nih.gov) (Table 2). Alignments were constructed in BioEdit Sequence Alignment Editor (Hall 1999) using ClustalW Multiple Alignment (Thompson et al. 1994) and checked for specificity against nucleotide expressed sequence tags in NCBI GenBank database. Primer specificity was checked using coral cDNA extracted from *Acropora millepora* at both the prawn chip and donut developmental stages and *Symbiodinium* clade C3 cDNA extracted from *A. aspera*. *Symbiodinium* C3 acpPC primers were designed to an expressed sequence tag library (Leggat et al. 2007). acpPC primers were designed and checked as per normalisation genes (Table 2).

An additional primer check and negative control to determine the effect of coral contamination on *Symbiodinium* relative expression was performed. A total of 30 ng of template was used with decreasing amounts of *Symbiodinium* cDNA template (30 ng to 0 ng) mixed with increasing concentrations of coral cDNA from the donut developmental stage (0 ng to 30 ng of coral). qPCR was performed according to the above protocol using β-actin, PCNA, acpPCSym_1 and acpPCSym_10 primers and relative gene

expression determined using the $\triangle\triangle C_t$ method (Livak and Schmittgen 2001).

Table 2: Normalisation genes and acpPC genes tested in qRT-PCR assays

Gene	Primers	Product size
β-actin	F1: TGG ACA ACG GAA GCG GAA TG B1: GCC AAC AAT GGA TGG GAA AAC T	80 bp
PCNA	F1: GAG TTT CAG AAG ATT TGC CGA GAT B1: ACA TTG CCA CTG CCG AGG TC	113 bp
18S rRNA	F3: GTC TAA CGC AAG GAA GTT TGAG B3: CAG GAC ATC TAA GGG CATC A	57 bp
acpPC Sym_10	F1: TTC GCC GAT GTG CCT AAT GG B1: TTC CTG GGA GAC TTC GCA GAA A	102 bp
acpPC Sym_1	F1: AGT GGA GTG AAC CAG GAA GCA A B1: AAC CAA TCG CAC CGA CCA AGA G	54 bp
acpPC Sym_9	F1: CGA ATG GAA GTT GGT GGT AAC B2: GTG CTC AAC CCA CTG TCT TTT	51 bp

Statistical analysis

Results were analysed using the geNorm (http://medgen.ugent.be/~jvdesomp/genorm/) statistical algorithm software to calculate gene expression stability (*M*) for the three normalisation genes tested. The gene or genes with the lowest *M* value is considered the most stably expressed (Vandesompele et al. 2002). All target samples collected on Day 7, which were determined to be the most stably expressed, and normalised to the two highest ranking normalisation genes were subsequently analysed using geNorm to identify a calibration sample. The three *Symbiodinium* acpPC gene expression profiles were analysed in the relative expression software REST© (http://www.gene-quantification.de/rest-2005.html) (Pfaffl et al. 2002). PCR efficiencies were calculated for each standard curve generated and threshold fixed at 0.0859 for all C_t calculations. This threshold value represented the average detection threshold across the 15 runs with the upper and lower bounds used to scan for an optimal threshold set between 1 and a value necessary to exclude background noise. Melting curve analysis was performed for each assay to check reaction specificity.

Results

Primer specificity and coral contamination

Specificity of primer design for *Symbiodinium* sp. normalisation genes was examined using coral cDNA isolated and transcribed from prawn chip and donut developmental stages, both early post fertilisation aposymbiotic stages. qPCR primers designed for clade C3 *Symbiodinium* and amplified according to either standard PCR conditions or qPCR conditions outlined above, failed to amplify coral cDNA. Glyceraldehye-3-phosphate dehydrogenase (GAPDH) qRT-PCR primers designed for *A. millepora* were included as a positive control with pawn chip cDNA. When tested with C3 *Symbiodinium* cDNA, single amplicons of expected size were detected (data not shown).

A possible confounding factor in using qPCR to quantify symbiont gene expression is the unknown contribution of host RNA. To test for this the relative expression of acpPCSym_1 and acpPCSym_10 was examined with increasing concentrations of coral cDNA. The concentration of *Symbiodinium* RNA (which would probably include approximately 10-20% host contamination (Leggat et al. 2007)) used in the assay was decreased from 100% to 0.5%, while the coral cDNA was increased. The expression of acpPCSym_1 and acpPCSym_10 was then compared across this concentration range. A variation from a relative expression of 1, when compared to a sample with 100% *Symbiodinium* cDNA (30 ng) would indicate an effect of coral contamination. Only when *Symbiodinium* cDNA composed 0.5% (0.15 ng) of the total cDNA was there a significant deviation ($p = 0.02$) from a relative expression value of 1 (Fig. 1). No fluorescence was recorded in samples without *Symbiodinium* template (30 ng of coral cDNA only).

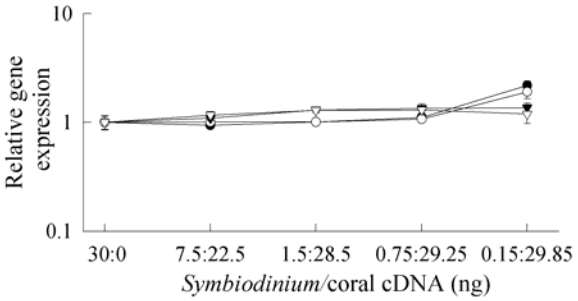

Figure 1: Effect of increasing the proportional coral cDNA concentration on relative expression of acpPCSym_1 normalised to β-actin (-●-) and PCNA (-o-) and acpPCSym_10 normalised to β-actin (-▼-) and PCNA (-▽-). Total template concentration is 30 ng for each sample and error bars indicating the standard error.

qRT-PCR efficiency

Amplification plots were analysed for the three normalisation and three *Symbiodinium* acpPC genes. Assay validations performed confirmed PCR efficiency and optimisation of procedure. Samples from Day 5 were removed from all expression profile analyses due to three samples being compromised prior to RNA extraction. Comparison of C_t values for each of the six genes on all sample days, including four additional templates run across multiple discs to ensure inter-disc comparability, confirmed minimal variation between PCR efficiencies, permitting comparison of genes and samples performed on different GeneDisc™-100 discs (data not shown).

C_t values for 18S rRNA were considerably lower than those of β-actin, PCNA and the three *Symbiodinium* acpPC genes across all sampling days (Fig. 2) indicating high transcript levels and the need for further optimisation of this gene.

Normalisation gene stability

β-actin (M = 0.467) was marginally more stable than PCNA (M = 0.498) with 18S rRNA the least stable (M = 0.687) under the specific experimental conditions used here. Target samples normalised to β-actin and PCNA from Day 7 of the experiment expressed the greatest stability. The most stably expressed target sample from Day 7 was used as the calibration sample and PCR efficiencies for each of the six genes from Day 7 were used to qualitatively compare the expression of the three acpPC genes subjected to varying light levels.

Relative gene expression levels for the three *Symbiodinium* light harvesting genes were calculated using the REST© method (Pfaffl et al. 2002) (Fig. 3). The profile of all three genes was similar when normalised to β-actin (Fig. 3a, c, e) with those samples shaded from light showing increased expression compared with light exposed samples. Light exposed samples exhibited minimal expression change across the nine day experiment and these results were replicated when acpPC genes were normalised to PCNA (Fig. 3b, d, f). acpPCSym_1 normalised to PCNA (Fig. 3f) were the only shaded samples not to increase expression.

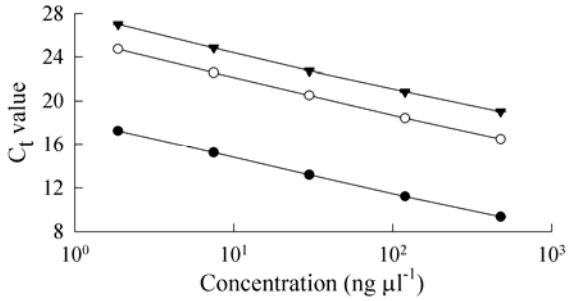

Figure 2: β-actin (-o-), PCNA (-▼-) and 18S rRNA (-●-) standard curves generated from amplification plots of serial four-fold dilution series. The plotted C_t is the average of triplicate samples and error bars (hidden by symbols) indicating the standard error.

Discussion

Three normalisation genes, β-actin, PCNA and 18S rRNA, were tested for stability in light manipulation experiments with *Symbiodinium* major light harvesting protein complexes (acpPC). Using the statistical algorithm software geNorm, β-actin was found to be marginally more stable than PCNA, and gene expression profiles for three acpPC genes normalised to β-actin and PCNA exhibited similar trends (Fig. 3).

PCNA was tested here rather than the more commonly used GAPDH to complement the use of β-actin and because synthesis of chloroplastic GAPDH is regulated over a diel cycle and exhibits greater than 50% identity in the region of overlap with the cytosolic isoform (Fagan et al. 1999). PCNA has been

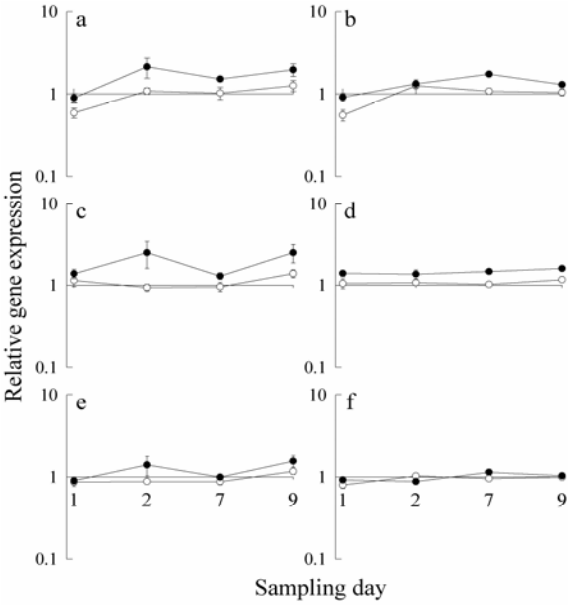

Figure 3: Expression profiles of *Symbiodinium* gene acpPCSym_10 (a, b), acpPCSym_9 (c, d) and acpPCSym_1 (e, f) normalised to β-actin (a, c, e) and PCNA (b, d, f) exposed to light (-o-) or shade from sunlight (-●-). Error bars represent the standard error for the averaged C_t values used to calculate relative expression for six biological replicates.

used as a normalisation gene in a range of animal species and humans (Schiller et al. 2003), is present in plant genomes (Suzuka et al. 1989) and has been isolated from a dinoflagellate where it was found not to significantly alter expression levels during cell cycle (Zhang et al. 2006), although this differs from other algal studies (example Wei et al. 2004).

Although using total RNA for normalisation is not always reflective of the mRNA fraction, it does enable the testing of 18S rRNA stability. In this study 18S rRNA was found to be unsuitable and would require additional optimisation. Compared with target mRNA transcripts the abundance of 18S rRNA is much higher (C_t value of approximately 10 compared to β-actin 17 and PCNA 19 indicating a 128-512 fold greater representation) (Fig. 2) and this causes difficulties when analysing data and determining the background baseline to subtract from the data (Vandesompele et al. 2002).

The overwhelming issue when using qRT-PCR with organisms such as *Symbiodinium* isolated from coral is whether varying RNA contamination influences the relative expression of the target genes. Results here suggest coral contamination is not a major factor and does not influence relative gene expression of *Symbiodinium* acpPC genes (Fig. 1) until transcript levels become very low, at which stage the variation between replicates increases. This suggests if valid normalisation genes can be identified for specific experimental conditions, the use of mixed

RNA populations will not confound results.

Microarray studies to date suggest dinoflagellate genes express small changes and that photosynthetic genes may only vary 2 – 3.4 fold (example Van Dolah et al. 2007). The identification of suitable normalisation genes is important if such minor changes in expression are to be detected and quantified. This study determined the stability of two normalisation genes and is an initial step towards validating a suite of genes with potential for use in *Symbiodinium*-coral gene expression work.

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High-performance Liquid Chromatographic Analysis of Photosynthetic Pigments in Corals: An Existence of a Variety of Epizoic, Endozoic and Endolithic Algae

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Abstract. Photosynthetic pigments of corals were analyzed by high-performance liquid chromatography (HPLC) to investigate the interrelationship between corals and their symbiotic algae, zooxanthellae (a dinoflagellate of the genus *Symbiodinium*). Coral samples were collected from Sesoko, Okinawa, Japan. HPLC analysis achieved the separation of more than 60 peaks of the pigments from more than 20 species of corals and 31 pigment types were identified. In addition to marker pigments of dinoflagellates, a variety of pigments including chlorophyll *b*,chlorophyll *d*, chlorophyll c_1, zeaxanthin, and lutein were detected. After brushing the surface of one of the common species of coral in the study area, *Montipora digitata*, to remove epizoic algae, the pigment composition of the coral was analyzed. However, the brushing treatment was not sufficient to remove most of the algae attached. These findings suggest that in addition to the pigments from symbiotic zooxanthellae, some of the pigments belong to epizoic and/or endolithic algae that grow in association with corals, such as cyanobacteria, green algae and diatoms. From these facts, corals are not only the host of symbiotic zooxanthellae, but also a community of diverse algae including cyanobacteria. This means that the coral host together with its associated algae may have an important role in terms of energy production for the whole reef ecosystem.

Keywords Photosynthetic pigments, HPLC, zooxanthellae, epizoic and endolithic algae, symbiosis

Introduction

The coloration of many reef-building corals is mainly derived from the photosynthetic pigments of their endosymbiotic, dinoflagellate algae of the genus *Symbiodinium*. The pigment composition of dinoflagellates is distinct from terrestrial plants and chlorophyte algae in containing chlorophylls c_1 and/or c_2, and the major xanthophylls peridinin or fucoxanthin (Kirk 1994). Dinoflagellates including *Symbiodinium* also contain the xanthophylls diadinoxanthin and diatoxanthin (Ambarsari *et al*. 1997), which are functionally equivalent to the photoprotective xanthophyll cycle pigments violaxanthin, antheraxanthin and zeaxanthin in terrestrial plants (Demmig-Adams & Adams 1996). *Symbiodinium* pigment profiles are dominated by chlorophyll *a* and peridinin, both of which comprise isomers in the HPLC eluant appearing as two or more peaks in HPLC chromatograms (Venn et al. 2006). All *Symbiodinium* also contain the pigments β-carotene and pheophytin *a* and additionally the minor xanthophyll dinoxanthin and the alteration product of diadinoxanthin, diadinochrome. These minor xanthophylls have been reported in HPLC studies of *Symbiodinium* (Kleppel et al. 1989; Ambarsari *et al*. 1997; Dove *et al*. 2006). Photosynthetic endolithic algae and cyanobacteria live within the skeletons of many reef-building corals. Under normal conditions, the green endolithic algae grow under less than 5% of the ambient photosynthetically active radiation (PAR) because of the absorbance of light by the zooxanthellae, coral tissues and the carbonate skeleton (Fine *et al*. 2005). However, there is little information concerning the diversity of such endolithic algae in corals.

In this study, we analyzed photosynthetic pigments of corals by HPLC to investigate the

interrelationship between the coral host and its epizoic and endolithic algae in order to understand their ecological role in the whole coral ecosystem.

Materials and Methods

Sampling of corals

Coral samples (see Table 1 for species list) were collected from Sesoko Island (26°38'54"N, 127°51'16"E) and Bisezaki (26°42'03"N, 127°52'35"E) located at the northern part of Okinawa, Japan. The collected corals were put into sterilized plastic bags, brought back immediately to the laboratory and stored at –30°C until treatment and pigment measurements.

Standard pigments

Chlorophyll *a*, pheophorbide *a*, α-carotene and β-carotene were purchased from Wako (Osaka, Japan). Chlorophyll c_2, chlorophyll c_3, divinyl chlorophyll *a*, alloxanthin, diadinoxanthin, fucoxanthin, 19'-butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin, lutein, peridinin, prasinoxanthin, violaxanthin, and zeaxanthin were obtained from DHI Water and Environment (Copenhagen, Denmark). Chlorophylls *a* and *b* were extracted from spinach (*Spinacia oleracea*) leaves and purified by sugar-column chromatography according to the method of Perkins and Roberts (1962). Pheophytins *a* and *b* were prepared by acid treatment of the respective chlorophylls as described previously (Shioi *et al*., 1983). Chlorophyll *d* was isolated and identified from the cells of *Acaryochloris* sp. or thalli of the red alga, *Carpopeltis crispate*, collected from Itoh City, Shizuoka, Japan.

Treatment of Montipora digitata

For removing the epizoics, living coral tissue surfaces were brushed gently using a toothbrush. Alternatively, coral tissues were removed by water pik treatment and centrifuged at 5000 xg for 10 min to separate zooxanthellae from the coral tissues.

Extraction of pigments from coral species

Frozen corals from field sampling were crushed into small pieces and then homogenized with 20 mL of cold 95% (v/v) methanol in a mortar for pigment extraction. After extracting the pigments with sonic treatment for 5 min, extracts were then filtered through a syringe filter (0.2 μm, Millex-LG, Millipore) to remove cell and skeleton debris. To avoid the shape distortion of earlier eluting peaks, methanol extract (1.0 mL) was mixed with 0.2 mL of distilled water just prior to injection according to the protocol described by Zapata et al. (2000). These extracted samples (200 μl) were immediately injected into the HPLC. All samples were prepared under subdued light and subjected to HPLC analysis within 5 min after extraction to avoid pigment destruction.

HPLC analysis

HPLC analysis was performed according to the method reported by Zapata et al. (2000). The HPLC system employed was model LC-10A equipped with degasser and column oven, using a Waters Symmetry C_8 column (4.6 x 150 mm). All apparatus was Shimadzu (Kyoto, Japan). Pigments were eluted at a flow rate of 1.0 mL per min at 25°C with a programmed binary gradient elution system according to the method. Solvents used were, A: methanol:acetonitrile: 0.25 M aqueous pyridine solution (50:25:25, by volume), and B: methanol:acetonitrile:acetone (20:60:20, by volume).

Pigment identification and quantification

Separated pigments were detected spectrophotometrically with a photodiode array detector, Shimadzu SPD-M10A, with an optical resolution of 1.2 nm, measuring from 320 to 720 nm and monitoring 5 channels of representative wavelengths at 410, 430, 440, 450 nm, and 663 nm. The wavelengths used to indicate the pigments were 410 nm for pheophorbide/ pheophytin *a* derivatives; 430 nm and 663 nm for chlorophyll *a* species; 440 nm for neoxanthin and violaxanthin; and 450 nm for chlorophyll *b*, chlorophyll *c* species and other carotenoids. Each peak was identified by comparison with HPLC retention times and absorption spectra of the standards and the data from photodiode array detection. A coelution of the standard pigment with a sample was done to assay more precisely, if necessary. Concentrations of each pigment were calculated from the standard curves, which were created for those 20 pigments from the relationships of concentrations and peak areas of HPLC using the appropriate wavelengths described above.

Results and Discussion

Pigment analysis of various coral species

As shown in elution profiles of representative corals (Fig. 1), HPLC analysis achieved the separation of more than 60 peaks of the pigments from more than 20 species of corals. Among them, 31 pigment species were

identified. The results of pigment identification from 20 coral species are summarized in Table 1. The pigment species extracted from corals were different in each coral, despite the fact that the corals used in this study were collected from similar environmental conditions. In addition to marker pigments of dinoflagellates, a variety of pigments including chlorophyll *b*, chlorophyll c_1, chlorophyll *d*, zeaxanthin, and lutein were detected. Interestingly, chlorophyll *d*, which is only present in prokaryotic *Acaryochloris*, was found from several species of corals. The absorption spectrum of chlorophyll *d* is shown in Fig. 2.

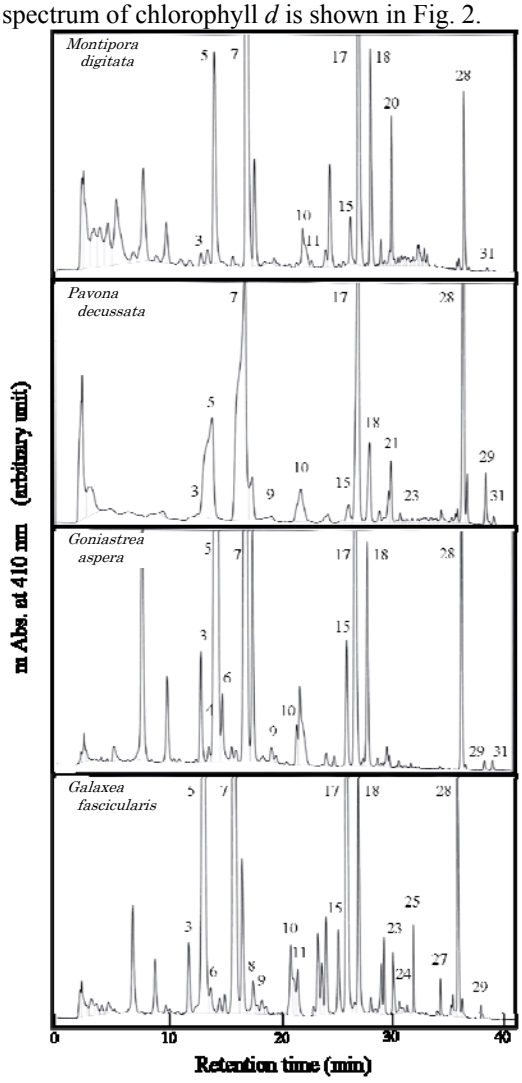

Fig. 1. Elution profiles of the photosynthetic pigments in representative coral species. HPLC conditions are described in the text. Peak numbers in elution profiles correspond to those in the identification table (Table 1).

Also, chlorophyll 684, which has a maximum absorption peak at 684 nm in the red band, was found in several species of corals. This is considered to be a degradation product of chlorophyll *a*. β-carotene is one of the marker pigments of dinoflagellates, but it was sometimes not detected. This is probably due to degradation of the pigment and it changing into its derivatives. As shown here, in addition to zooxanthellar pigments, various photosynthetic pigments from different algae were detected in corals. This finding shows that some of the pigments are probably due to the presence of epizoic and/or endolithic algae, growing on the coral surface or inside the coral skeleton, such as green algae, diatoms and cyanobacteria.

Pigment analysis of treated M. digitata

To examine whether algae are attached to the coral surface or not, brushing and water pik treatment of the coral were carried out. After brushing *M. digitata*, to remove epizoic algae, the pigment composition of corals was analyzed in the same manner. As shown in Table 2, even after brushing, similar types of photosynthetic pigments from different algae were detected in corals, in addition to zooxanthellar pigments, indicating that most of the epizoics could not be removed. Similar results were also obtained from water pik treatment. These facts show that epizoic algae are firmly attached and/or live inside the coral tissue.

Conclusion

In this study, we showed that some of the pigments detected by the HPLC technique in corals belong to various epizoic, endozoic and/or endolithic algae growing in association with the coral host. Moreover, these algae were unable to be removed by brushing treatment. Therefore, corals are not only hosts for their zooxanthellae, but also for various other algae. This means that the coral host and its algae might have an even more important role in the primary production of the whole ecosystem than otherwise suspected. Further research is necessary to investigate not only the relationship between corals and *Symbionidium*, but also interrelationships among coral hosts and their algal community.

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Table 1. Identification of photosynthetic pigments in 20 species of corals

Peak No.	t_R (min)	Maxima in eluant (nm)			Pigment	*Montipora digitata*	*Montipora aequituberculata*	*Montipora peltiformis*	*Montipora grisea*	*Montipora sammarensis*	*Acropora latistella*	*Pavona decussata*	*Favites chinensis*	*Favia favus*	*Goniastrea aspera*	*Cyphastrea serailia*	*Platygyra daedalea*	*Leptastrea purpurea*	*Leptastrea transversa*	*Astreopora myriophthalma*	*Symphyllia recta*	*Psammocora contigua*	*Galaxea fascicularis*	*Porites lutea*	*Porites cylindrica*
1	8.329	465	601	650	Chlorophyllide *b*			+	+					+	+	+	+		+	+					
2	11.050	451	584	633	MV Chlorophyll c_3																		+		
3	12.893	429	618	665	Chlorophyllide *a*	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
4	13.425	439		629	Mg DVP																				
5	14.132	452	584	634	**Chlorophyll c_2**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	14.869	446	583	631	Chlorophyll c_1	+	+	+	+		+	+		+	+		+	+	+	+	+	+	+	+	+
7	17.019	474			**Peridinin**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	18.354	411		665	Pheophorbide *a*		+	+	+		+		+	+	+	+		+	+	+	+	+		+	+
9	19.463	454	473		Uriolide	+						+											+		
10	22.026	461			Fucoxanthin	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	22.799	418	438	467	Neoxanthin	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	
12	23.832	456			Prasinoxanthin																				
13	23.951	418	441	470	Violaxanthin			+	+					+	+	+	+	+						+	
14	24.000	446	469		19'-Hexanoyloxyfucoxanthin			+	+					+	+	+	+	+	+		+	+		+	
15	24.078	478			Astaxanthin	+						+													
16	26.302	405	429	457	Diadinochrome	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
17	27.080	425	447	477	**Diadinoxanthin**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	28.135	417	442	470	**Dinoxanthin**	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
19	29.535	428	451	478	Alloxanthin																				
20	30.002	427	452	480	Diatoxanthin	+			+											+				+	
21	30.187	420	448	472	Monadoxanthin							+													
22	30.506	427	453	480	Zeaxanthin		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+		+	+
23	31.525	422	446	474	Lutein	+		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	32.222	427	453	684	Chlorophyll 684				+										+				+		+
25	33.511	457			Siphonein			+	+					+	+	+	+	+	+	+			+	+	
26	34.806	458	646	691	Chlorophyll *d*				+	+	+														
27	35.115	461	608	649	Chlorophyll *b*			+	+				+	+	+	+	+	+	+	+	+	+	+	+	
28	36.492	431	616	662	**Chlorophyll *a***	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	38.536	406	503	665	Pheophytin *a*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	39.012	422	446	475	α-Carotene				+																
31	39.195	425	451	478	**β-Carotene**				+	+		+		+			+							+	+

Bold, Marker pigments of dinoflagellates

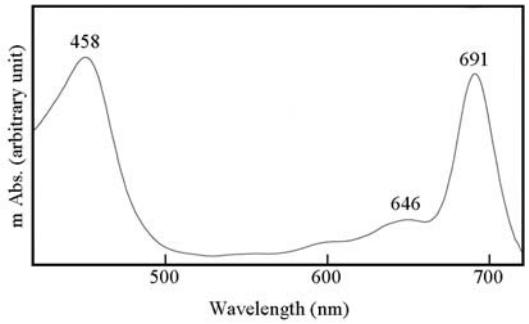

Fig. 2. Diode array absorption spectrum of chlorophyll *d* extracted from *Montipora sammarensis*. Separation and analysis of the pigment are described in the text.

Table 2. Identification of photosynthetic pigments in *Montipora digitata*

Peak No.	t_R (min)	Maxima in eluant (nm)			Pigment	No treatment n = 7	Wash n = 7	Water pick n = 7
3	12.599	431	622	665	Chlorophyllide *a*	7	7	0
5	13.906	452	585	634	**Chlorophyll c_2**	7	7	7
6	14.570	445	581	629	Chlorophyll c_1	7	7	7
7	16.740	474			**Peridinin**	7	7	7
8	18.980	410	507	668	Pheophorbide *a*	0	0	7
9	19.388	452	475		Uriolid	7	7	0
10	22.099	449			Fucoxanthin	7	7	7
11	22.749	413	439	468	Neoxanthin	7	7	0
16	26.324	408	429	457	Diadinochrome	7	7	7
17	27.151	422	446	474	**Diadinoxanthin**	7	7	7
18	28.228	418	441	469	**Dinoxanthin**	7	7	7
20	30.010	429	452	479	Diatoxanthin	7	7	7
23	31.680	422	448	477	Lutein	0	0	7
26	34.883	458	646	691	Chlorophyll *d*	2	1	0
27	34.970	458		646	Chlorophyll *b*	1	0	0
28	36.695	431	616	662	**Chlorophyll *a***	7	7	7
29	38.787	408	504	666	Pheophytin *a*	5	6	7
31	39.764	448	477		**β-Carotene**	0	0	5

Peak numbers correspond to those of Table 1. **Bold**, Marker pigments of dinoflagellates; Under line, Values changed.

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Unravelling coral photoacclimation: *Symbiodinium* strategy and host modification

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Abstract. Light is often the most abundant resource within the nutrient poor waters surrounding coral reefs. Consequently, zooxanthellae (*Symbiodinium spp.*) must continually photoacclimate to optimise productivity and ensure coral success. To accurately assess *Symbiodinium* photoacclimation *in situ*, differences in acclimation strategies and bio-optical signatures need to be characterised between genetic types of *Symbiodinium.* Using a systematic series of laboratory experiments, eight types of *Symbiodinium* were cultured and examined using techniques such as active (FIRe) fluorescence, Photosystem I (PSI) and II counts and spectrophotometry. Two key 'strategies' of photoacclimation are known to exist amongst microalgae: a preferential modification of the light harvesting antennae (σ-based) or of the reaction centre bed (n-based) for PSII and/or PSI. Our measurements demonstrated that acclimation strategies employed by *Symbiodinium* were highly varied between algal type but despite this variability, many optical signatures were conserved. Acclimation strategies of intact *Acropora formosa* and *Seriatopora caliendrum* at two light levels were further examined using fluorescence and optical signatures to determine host contribution to acclimation. Overall, our results demonstrated that (1) biophysical (active fluorescence, photosystem-specific) but not bio-optical signatures were highly variable between algal types; consequently, bio-physical signatures that are altered by an adaptation of the algal community structure may be misinterpreted as photoacclimation and (2) host acclimation and modification of the light environment plays a key role in *Symbiodinium* photoacclimation.

Key words: *Symbiodinium,* chlorophyll *a* fluorescence, photoacclimation, absorption

Introduction

In the oligotrophic waters surrounding coral reefs, optimisation of photosynthesis is crucial for the success of the symbiotic partnership between the microalgae (*Symbiodinium spp*) and the host coral. This is achieved through the process of photoacclimation (phenotypic modification of the photosynthetic apparatus in response to changes in light availability). However, this phenomenon is not ubiquitous for all coral-*Symbiodinium* assemblages.

Multiple genetic types of *Symbiodinium* exist, and have been categorised into 8 major clades, A-H (Coffroth and Santos 2005). These clades can be further split into sub-clades according to the ribosomal Internal Transcribed Region 2 (ITS2) (LaJeunesse et al. 2001, Robison and Warner 2006). It was initially assumed that different clades conformed as 'eco-types' such as thermally tolerant or sensitive, but recent evidence has shown that variability exists within each clade (Iglesias-Prieto et al. 1994, 1997, Tchernov et al. 2004, Robison and Warner 2006, Hennige et al. 2009).

A coral species may comprise of a mixed community of *Symbiodinium* types, including different clades as well as subclades (LaJeunesse et al. 2004, Goulet 2006). The importance of this for coping with environmental change (including light) is debated (Baker 2001, Hoegh-Guldberg et al. 2002) but centres around whether corals can adaptively bleach (expel sub-optimal zooxanthellae and 'uptake' suitable types), or whether the *Symbiodinium* community already *within* the coral will 'shuffle' in dominance as conditions favour growth of one type over others. Regardless of potential benefits related to 'hosting' multiple *Symbiodinium* types, the presence of multiple types complicates interpretation of any *in hospite* photoacclimation study where the genetic identity of *Symbiodinium* is unknown, as genetic variability may 'mask' potential photoacclimation responses (Hennige et al. 2008, 2009).

Several photoacclimation strategies are known to exist for microalgae (Falkowski and Owens 1981, Suggett et al. 2007): cells can preferentially undergo changes to the light harvesting antennae

(photosynthetic unit, PSU, size) or of the reaction centre pool size (PSU number). This 'choice' varies, but recent work on phytoplankton has identified two strategies which conform to environmental conditions such as nutrient and light availability (Moore et al. 2006, Warner et al. 2006, Six et al. 2008). The two strategies involve either changes to PSU size or number and were termed σ versus n–type acclimation strategies respectively (Six et al. 2008). N-type acclimation tends to be found in generalist microalgae species which live in variable light environments and are not nutrient limited. Conversely, σ-type acclimation is better suited to uniform or low light environments (Six et al. 2008).

Methods for assessing photoacclimation are often destructive, so recent *in situ* coral photoacclimation studies have turned towards non-invasive techniques, such as chlorophyll *a* (chl *a*) fluorescence and optical reflectance. Chl *a* fluorescence can be used to infer photosynthetic efficiency and effective cross section-absorption (σ) of *in hospite Symbiodinium* (Gorbunov et al 2001). Consequently, studies have used chl *a* fluorescence to assess coral photoacclimation across environmental gradients (Ralph et al. 1999, Hennige et al. 2008). However, many studies, past and present, do not account for the community composition of *in hospite Symbiodinium*; consequently, studies often assess photoacclimation on the basis that the genotype of *Symbiodinium* does not change.

These coral-*Symbiodinium* assemblages are often considered as a single entity – the holobiont. Since some hosts only associate with certain *Symbiodinium* types, are some holobionts more suited to certain environments than others? To answer this, a better understanding is needed of the photobiological variability between different *Symbiodinium* types, and also how the host can play a role in modifying the internal light environment to optimise *Symbiodinium* productivity.

This study addressed 2 primary objectives: (1) to categorise variability between different *Symbiodinium* types using bio-optical and bio-physical techniques; and (2) to assess whether host acclimation and modification plays a role in *Symbiodinium* photoacclimation.

Material and Methods

Isolated Symbiodinium - Eight *Symbiodinium* types identified using the ribosomal ITS2 region; A1, A1.1, A2, A3, B1, B1*, B1** and F2 (Table 1) were cultured in 2L flasks at 26°C at two photon flux densities (PFD, 100 (LL) and 650 (HL) μmol photons m^{-2} s^{-1}) on a 14:10 light: dark cycle (see Hennige et al. 2009). Cultures were semi-continuous in artificial seawater media ASP-8A, (Provasoli et al. 1957) and were bubbled gently with air passed via a carbon column to keep pH constant (ca. 8.3). In exponential phase, aliquots of 500 ml were gravity filtered to concentrate material for subsequent measurements (Suggett et al. 2007). Triplicates were taken from sequential generations.

Table 1: *Symbiodinium* type according to ITS2 region, the host and region they were isolated from (adapted from Hennige et al. 2009)

Algal type	Host	Host origin
A1	*Cassiopeia xamachana*	Florida
A1.1	*Condylactis gigantea*	Jamaica
A2	*Montastrea spp.*	Florida
A3	*Tridacna maxima*	Palau
B1	*Aiptasia pallida*	Bermuda
B1*	*Aiptasia puchella*	Hawaii
B1**	*Acropora spp.*	Aquarium (UK)
F2	*Meandrina meandrites*	Jamaica

Corals – small fragments (ca. 6 cm) of *Seriatopora caliendrum* and *Acropora formosa* (from London Aquarium) were maintained in separate 250 ml water jacketed vessels under a light: dark cycle of 12: 12 at 26°C for 12 weeks. Media was circulated through the vessels at 40 ml $hour^{-1}$. Each vessel was integrated with an inflow, an outflow and an aerator to provide water exchange and circulation. A planktonic food supplement was added to the media reservoir to provide a constant external nitrogen source to all vessels. There were two light treatments; low light, ca. 40, and high light, ca. 170 μmols photons m^{-2} s^{-1}. These light levels represent reef depths in Indonesia of ca. 20 and 10 m respectively (June – September).

N versus σ strategies– Photosynthetic unit sizes, which are used to assess σ–type acclimation, were defined as the ratio of chl *a* to reaction centres in PSI (RCI) or RCII (Suggett et al. 2007, Hennige et al. 2009), and are considered to be indicative of the concentration of functional reaction centres (RCs). Cell counts were determined using a haemocytometer, and subsequently used to calculate n–type acclimation as mol RCII(I) $cell^{-1}$.

To assess chl *a* fluorescence of *Symbiodinium*, a Fluorescence Induction and Relaxation (FIRe) fluorometer (Satlantic), which generates single turnover (ST) and multiple turnover (MT) fluorescence transients was used (Fig. 1). A ST protocol is where the primary electron acceptor Q_A, is fully reduced through a simultaneous single turnover event of all PSII reaction centres (Suggett et al. 2008).

A U-3000 spectrophotometer with ϕ–60 integrating sphere (Hitachi) was used to determine sample optical density as outlined in Suggett et al. (2007) and Hennige et al. (2009).

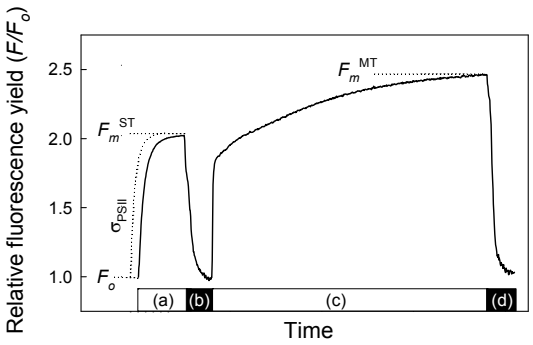

Figure 1: Example fluorescence induction trace using a FIRe fluorometer. Minimum fluorescence, F_o, maximum fluorescence (single turnover) F_m^{ST}, multiple turnover F_m^{MT}, and effective cross-section absorption of PSII (σ_{PSII}) are illustrated. Letters a - d refer to fluorescence induction stages along a time scale; a) describes the rise in fluorescence following a 100 µs single turnover (ST) event, b) is the 500 ms relaxation stage following the ST event, c) is the fluorescence rise from a multiple turnover (MT) flash over 600 ms and d) is the relaxation stage following the MT event over 1 s (adapted from Hennige et al. 2009)

To assess host and freshly isolated zooxanthellae absorption, coral tissue was removed from the coral skeletons using a waterpik. The slurry was separated into host and zooxanthellae portions by centrifuging twice at 1500***g*** for 15 min (Levy et al. 2003). The zooxanthellae pellet was re-suspended in a known quantity of seawater and the supernatant was regarded as the host fraction for subsequent spectrophotometry.

Surface area of the coral skeletons was quantified using tin foil and Image Tool analysis (UTHSCSA). Chlorophyll content of each sample and per colony was calculated using methanol and a spectrophotometer in accordance with Porra et al. (1989).

Results

Photophysiology - under steady state growth, significant variability was observed between algal types in F_v/F_m^{ST} ($F_{7,16} = 59.0$ and 35.8, $p < 0.001$ for LL and HL respectively) and σ_{PSII} ($F_{7,16} = 15.62$ and 3.81, $p < 0.05$ for LL and HL respectively), (see also Fig. 2). Mean values for F_v/F_m^{ST} and σ_{PSII} were ca. 25% and 5% lower under HL than LL respectively, but the magnitude of change was type-dependent.

Photosynthetic unit size and concentration- values of photosynthetic unit (PSU) 'size', chl *a*: RCII (I) used to assess σ–type acclimation, were highly variable with algal type (Data not shown). Most algal types exhibited a decrease in chl *a*: RCII between LL and HL but this was highly variable; algal type A1.1 exhibited an increase whilst type A2 exhibited no change in chl *a*: RCII (Table 3).

Changes to chl *a*: RCI between LL and HL were also highly variable between type, either increasing (A1.1), decreasing (B1, F2) or exhibiting little or no change (A1, A2, A3, B1*, B1**). Similarly, the cellular quotas (n-type acclimation) of both RCI and RCIIs were highly variable between algal types and upon an increase in growth PFD, most types decreased cellular RC content (Table 3). F2 was the only type to significantly increase cellular RC content from low to high growth PFD. Consequently, RC stoichiometry of PSII to PSI (RCII: RCI) did not vary in a consistent pattern for algal type or growth PFD.

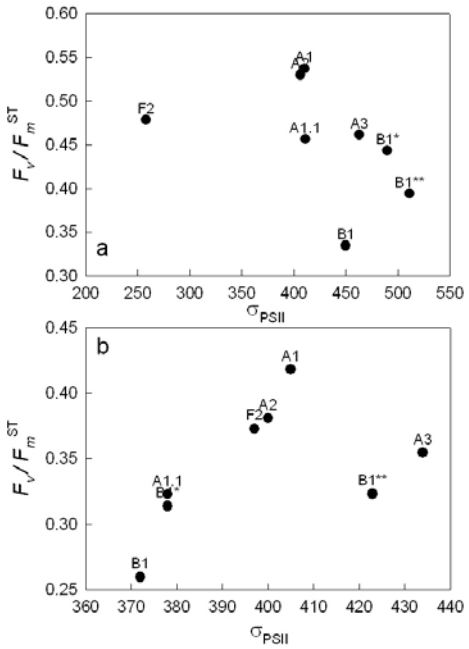

Figure 2: Maximum photochemical efficiency (F_v/F_m^{ST}) and effective cross section absorption of PSII (σ_{PSII}) of all *Symbiodinium* types under LL (a) and HL (b). For absolute values refer to Hennige et al. 2009

Table 3: Percentage difference in RC cell^{-1} (n) and chl *a* RC^{-1} (σ) for PSII and PSI between low and high light replicates. For absolute values refer to Hennige et al. 2009. Significant differences between LL and HL cultures are denoted with * (t-test, $p < 0.05$)

Algal Type	PSII		PSI	
	Number (RCII cell^{-1})	Size (chl *a* RCII^{-1})	Number (RCI cell^{-1})	Size (chl *a* RCI^{-1})
A1	-41.79	-17.46	-56.43*	10.27
A1.1	-72.62*	57.96*	-61.22*	11.55
A2	-57.08*	0.22	-46.69*	-36.24*
A3	14.93	-14.51	11.08	-3.68
B1	-73.90*	-44.53*	-78.96*	-31.18
B1*	-61.98*	-9.36	-46.41*	-35.70*
B1**	-6.9	-36.76	-51.9*	11.29
F2	34.95*	-57.07*	60.68*	-63.95*

Optical absorption, a^* (m^2 mg chl a^{-1}), was variable between algal type at LL and HL (Fig. 3), but was similar in shape for all algal types. Consequently, a^* was significantly correlated with pigment concentrations (Hennige et al. 2009). a^* at HL was

higher than at LL (Fig. 3) but percentage increase in absorption was type dependent.

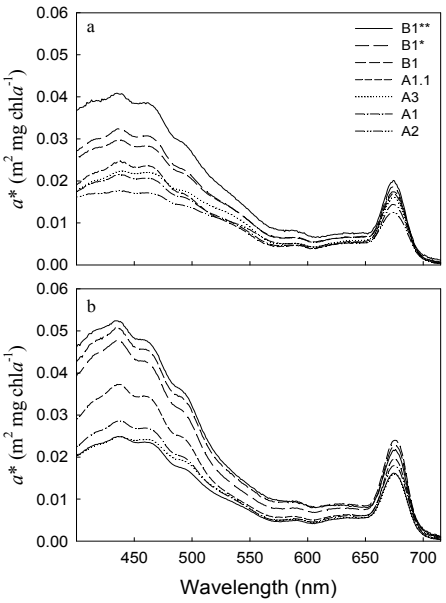

Figure 3: Optical absorption normalised to chlorophyll *a*, *a** (m^2 mg chl a^{-1}), from 400 to 715 nm for all *Symbiodinium* types except F2 under (a) LL and (b) HL.

Partitioning bio-optical signatures –freshly isolated zooxanthellae bulk absorption (Fig. 4a,c) was comparable to cultured *Symbiodinium* optical absorption (Fig. 3). Host tissue absorbed primarily at ca. 400 nm and decreased linearly to ca. 700 nm (Fig. 3a,b). Host absorption presented here was comparable to previous studies (Enriquez et al. 2005) but was higher in magnitude. Slurry absorption in Fig. 4a, was presented as the sum of both host and zooxanthellae fractions, which could differ by between ca. 2 – 10 % in magnitude of the measured slurry optical absorption.

When host fractions from *S. caliendrum* and *A. formosa* were compared between low light (LL) and high light (HL), HL corals had higher absorption. Additionally, *A. formosa* had an absorption peak (at both light levels) at ca. 500 nm (Fig. 4b) which was not present in *S. caliendrum* and represents the presence of a fluorescent protein, (confirmed with spectral fluorescence data (data not shown)). However, *S. caliendrum* host absorption was higher than *A. formosa* between 400 and ca. 460 nm.

Zooxanthellae bulk absorption (Fig. 4c) was influenced more by host species than light regime, since between LL and HL for each coral species, there was negligible change in zooxanthellae absorption (m^2 mg chl a^{-1}). Zooxanthellae isolated from *S. caliendrum* had higher absorption than *A. formosa* zooxanthellae (Fig. 4c).

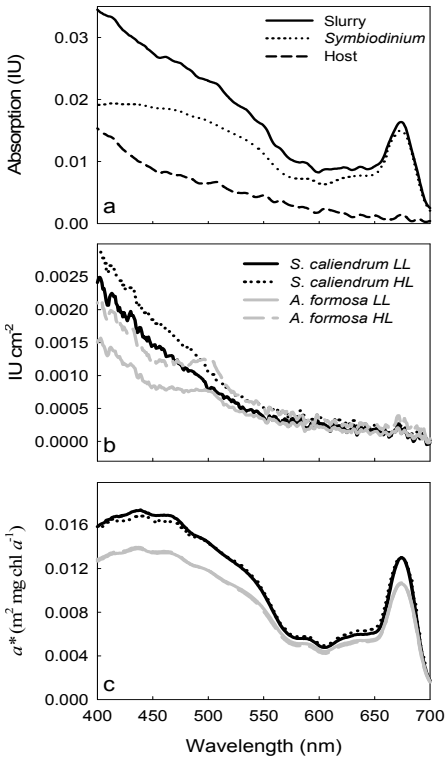

Figure 4: Example absorption characteristics (Instrument Units) of *S.caliendrum* tissue (slurry) and its constituents removed from the coral skeleton (a). Absorption of host tissue (IU cm^{-2}) from *S. caliendrum* and *A. formosa* from LL and HL conditions (b) and of isolated zooxanthellae (m^2 mg chl a^{-1} (c))

Discussion

Substantial variability was observed both in and between algal types in their n and σ-type photoacclimation strategies, consistent with previous studies (Iglesias-Prieto and Trench 1994, Hennige et al. 2009). The variability in bio-physical signatures such as F_v/F_m^{ST} was often larger between types than between environmental conditions. Consequently, if F_v/F_m^{ST} is used to assess photoacclimation across environmental gradients, *Symbiodinium* type must be accounted for to prevent an adaptation of the algal community being misidentified as photoacclimation.

Bio-optical signatures were conserved between types and varied according to pigment concentrations. Since *a** consists of photosystem II and I absorption,

$$a^*(\text{optical}) = a^*\,\text{PSII} + a^*\,\text{PSI} \qquad [1]$$

and optical absorption is controlled by both PSU size and number, we can consider *a**(optical) as

$$a^*(\text{optical}) = [\sigma \cdot n]\text{PSII} + [\sigma \cdot n]\text{PSI} \qquad [2]$$

Since *a** is conserved across *Symbiodinium* types, and n-type strategies are dominant across types, changes in σ between photosystems must therefore balance

changes in n. The predominant n-type strategy in *Symbiodinium* suggests the role of a generalist algal strategy when subject to varying light intensities, which are not nutrient limited (Six et al. 2008). Some types (A1.1, B1's and F2) also exhibited a σ-type strategy in addition to n (Table 3), which may confer additional benefits (Hennige et al. 2009).

Results here enabled direct comparison between zooxanthellae isolated from different coral hosts; the *internal* light environment for *S. caliendrum* was lower than that in *A. formosa.* Increased host absorption by *S. caliendrum* between 400 and 500 nm may have contributed to this. A lower light environment would promote the observed increase in algal absorption to optimise photosynthesis (Fig. 3c). These differences between host absorption could be attributed to increased host tissue volume per unit area as noted by Anthony et al. (2003), or by differences between host pigment content. The presence of a possible green fluorescent protein is noted at ca. 510 nm in *A. formosa* (Fig. 4b). However, skeletally enhanced light (not measured), may also have differed between host species and contributed to internal light variability (Enriquez et al. 2005).

In summary, both parts of the holobiont; the *Symbiodinium* and the host, are crucial to overall coral acclimation. However, the contribution of both fractions to acclimation is still not fully quantified. Importantly, bio-physical approaches need additional genetic identification (as opposed to bio-optical approaches) of the *Symbiodinium* to assess photoacclimation, consequently meaning that grouping algal clades as 'eco-types' may not be suitable. The host modification of internal light environment also differs between coral species, and in some cases may cause as much variability between species as between external light environments. Consequently, future photoacclimation studies will have to account for both *Symbiodinium* and host contribution to unravel coral photoacclimation.

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Assessing ultraviolet radiation-induced DNA damage and repair in field-collected *Aiptasia pallida* using the comet assay

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Abstract. Ultraviolet radiation (UVR)-induced DNA damage and repair in freshly-collected *Aiptasia pallida* from Walsingham Pond, Bermuda were examined using the comet assay. In addition, animals were screened for the presence of mycosporine-like amino acids (MAAs). We found that field anemones produce relatively large quantities of four MAAs (mycosporine-2-glycine, mycosporine glycine, shinorine and Porphyra-334). Additionally, field anemones were capable of fully repairing DNA damage incurred during a 12-h exposure to UVR and PAR. It appears that *A. pallida* initiates nucleotide excision repair (NER) during the first 2 h of recovery in the dark. The majority of DNA strand breaks (SB) formed from NER are repaired within an 8-h recovery period. These results suggest that the ability of *A. pallida* to repair DNA damage and / or protect itself from the detrimental effects of UVR are important factors for its survival.

Keywords: Comet assay, DNA damage, *Aiptasia pallida*, Ultraviolet radiation.

Introduction

Tropical littoral zones experience some of the highest doses of ultraviolet radiation (UVR) on Earth (Frederick et al. 1989; McKenzie et al. 2003). Consequently, sedentary marine invertebrates in these habitats are exposed to biologically harmful levels of ultraviolet-A (UVA, 320-400nm) and ultraviolet-B (UVB, 280-320nm) (Shick and Dunlap 2002). Exposure to UVA and UVB can detrimentally affect survival, growth, and reproduction of shallow-water marine invertebrates from oxidative stress that results in structural damage to proteins and lipids and physiological impairment at the cellular level (Stochaj et al. 1994).

DNA damage is arguably the most harmful effect of UVR. Exposure to UVR generates the formation of cyclobutanepyrimidine dimers (CPDs) and 6-4 photoproducts (Ananthaswamy 1997).

DNA lesions are repaired via enzymatic mechanisms such as nucleotide excision repair (NER), base excision repair (BER) (Kantor 1995) and photolyase activity (photoreactivation).

In addition to UVR exposure, single strand breaks are caused during oxidative stress when reactive oxygen species attack the backbone of DNA. Double strand breaks, which are the most severe form of DNA damage, can occur if two single strand breaks are nearby one another (Kantor 1995).

Symbiotic marine cnidarians are especially susceptible to these stresses in that UVR often co-occurs with intracellular hyperoxic stress (due to photosynthesis by zooxanthellae) and elevated environmental temperatures. These factors contribute synergistically to create oxidative stress and have been linked to bleaching events (Lesser 1997).

Organisms employ behavioral and physiological adaptations to defend themselves against the harmful effects of solar radiation. The most commonly reported defense against UVR exposure is the production of mycosporine-like amino acids (MAAs).

While many studies show that organisms produce MAAs in response to UVR exposure, few have demonstrated a link between DNA damage and MAA concentration.

The use of the comet assay has been suggested as a sensitive and rapid technique for the detection of DNA strand breaks caused by genotoxins in the environment and has recently been adapted for use in studies with cnidarians (Michelmore and Hyatt 2004). Rinkevich et al. (2005) were the first to employ the comet assay specifically to investigate UVR exposure and DNA damage in corals (*Stylophora pistillata*).

Here we present evidence that field-collected specimens of *Aiptasia pallida* efficiently repair DNA damage incurred during a 12-h exposure to photosynthetically available radiation (PAR) and UVR.

Material and Methods

Collection of A. pallida

Specimens of *A. pallida* were collected at 15:00 hrs from Walsingham Pond, Bermuda and transported to the Bermuda Institute of Ocean Sciences (BIOS) on the 19th June 2007. Surface water temperature and salinity readings at the time of collection were 26 °C and 37 ppt, respectively. Upon return to the BIOS station the anemones were maintained on an indoor seawater table for no longer than 16 hrs before being exposed to PAR and UVR.

Experimental design

Field-collected anemones were randomly transferred into four 100 x 50 mm Pyrex crystallization dishes (n=5 per dish) and maintained in an outdoor seawater flow-through tank. In addition to the 4 UVR exposed dishes, a control group (n=3) was included which was not exposed to UVR. For the control, individual anemones were placed in a 30 ml beaker and nested into the crystallization dish. To block UV transmission the control dish was completely covered with 3 layers each of Mylar film (a UVB blocker) and C3a film (a UVA blocker).

Due to shading from mangrove trees at Walsingham Pond, light readings were lower than those at BIOS. At Walsingham Pond UVB measured 1.6 W m^{-2} and PAR 1104 µmol photons m^{-2} s^{-1}, whereas at the BIOS station unshaded UVB measured 2.51 W m^{-2} and PAR 1742 µmol photons m^{-2} s^{-1}. Therefore, animals were exposed to a reduced level of PAR and UVR to better represent the radiation found in their typical habitat of Walsingham Pond (Fig. 1).

In order to reduce PAR and UVR the holding tank which contained dishes of anemones was covered with a sheet of Solacryl® SUVT (Spartech Polycast) (which allowed UV and PAR transmission) and one layer of black fiberglass screen.

Anemones were subjected to 12 hrs of PAR and UVR (Fig. 1); receiving an approximate UVB dose of 24 kJ m^{-2}. Sunrise was at approximately 06:00 hrs and sunset not until 20:00 hrs. To cease exposure after 12 hrs, we covered the flow-through tank with 2 layers of neutral density filter to mimic sundown at 18:00 hrs. In this way, samples remained in the dark after PAR and UVR exposure for recovery periods of 0, 2, 4, 6, and 8 h. There were 4 replicate anemones for each recovery time. Animals were randomly chosen by removing 1 individual each from the 4 UV-exposed dishes. These individuals were processed immediately to determine the extent of DNA damage incurred via the comet assay. A control group (n=3) was also immediately processed after 12 h of PAR but in the absence of UVR.

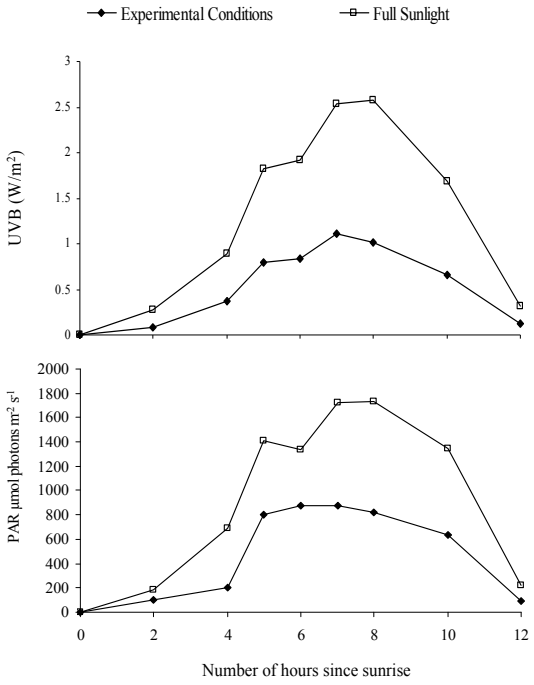

Figure 1: Light regime on 20 June 2007 at the BIOS. Field-collected symbiotic *A. pallida* were subjected to reduced PAR and UVR compared to full sunlight to mimic conditions observed at Walsingham Pond, Bermuda.

Assessing DNA damage using the comet assay

Host nuclei were isolated immediately following UVR exposures and subsequent recovery periods. This process occurred in a darkened room with no fluorescent lighting.

Anemones were carefully blotted with a Kimwipe to remove excess mucus then transferred to a Potter-Elvehjem homogenizer and homogenized in 1 ml of buffer (1x PBS + 0.25% sucrose; 100 µM EDTA (ethyl-enediaminetetra-acetic acid) pH 8; and 10% DMSO (dimethyl sulfoxide). Homogenization was achieved after 7 strokes of the Teflon pestle while rotating it 360° during each stroke.

The homogenate was passed through a cell strainer (50 µm) and carefully transferred using a Pasteur pipette onto a chilled 20% sucrose pillow to form a second layer. The cell suspension and pillow were centrifuged at 1340*g* for 2 min, which pelleted algal cells. The nuclei formed a surface layer on the pillow, which was transferred into a clean centrifuge tube and centrifuged at 4000*g* for 2 min to pellet the nuclei. The isolated nuclei were re-suspended in 0.7% low melting-point agarose.

Gel-bond™ slides were pre-coated with 150 µl 1% normal melting-point agar and covered with a glass cover-slip. Once the gels solidified the cover-slips were removed and 70 µl of nuclei suspension were layered onto the pre-coated Gel-bond™ slides, a new cover slip was added and the agarose containing

the nuclei allowed to solidify on ice in the dark for at least 15 min.

The cover-slips were removed and the slides transferred to a lysis solution (10% DMSO, 2.5 M NaCl, 100 mM EDTA, 10 mM Tris; pH 10) on ice and in the dark for 10 min. Following lysis the slides were immersed in alkaline unwinding buffer (300 mM NaOH, 1 mM EDTA; pH >10) on ice and in the dark for 5 min to unwind super-coiled DNA. Comet formation was achieved via electrophoresis in alkaline unwinding buffer at a constant amperage of 300 mA for 5 min.

Following electrophoresis, the slides were neutralized with three rinses in 0.4 M Tris; pH 7.5 for 10 min each, dehydrated with 95% ethanol for 5 min and allowed to air dry at room temperature for at least 24 h.

Dried gels were stained with 40 µl ethidium bromide solution (20 µg/ml)and examined under 400x magnification using a Zeiss Axioskopp epifluorescent microscope (excitation filter 510-560 nm green light, barrier filter 590 nm).

Digital images of the nuclei were taken with a Nikon, Coolpix 995 and analyzed using TriTek Comet Score™ (Freeware v.1.5). At least 50 nuclei per animal were analyzed for tail length (µm), and % DNA in tail and expressed as tail moment (i.e. the product of the two measurements). Results are expressed as means ± standard error of the mean (SEM).

MAA analysis

A sample of anemones (n=3) was immediately extracted for MAA content. Anemones were blotted dry and then homogenized in 1 ml 100 % methanol. A 100 µl sample was taken and diluted with deionized water for protein analysis (Bradford, 1976). The remainder was extracted for MAAs.

The homogenate was transferred into a microcentrifuge tube and extracted for 1 h in the dark at 4 ºC. Following centrifugation the supernatant was withdrawn and set aside. The pellet was extracted two more times. The pooled supernatant of individual anemones was passed through a SepPak C18 cartridge to remove pigment and lipid contaminants.

A spectrophotometric scan (280-400 nm) of the pooled extract (5x dilution) for each individual was performed on a Spectronic Genesys 5 UV-Vis to ascertain the presence of UV absorbing compounds. The pooled extracts were then evaporated to dryness using a Savant Speed-Vac SC100 and stored at -20 ºC until the samples were analyzed for MAA content via liquid chromatography followed by photodioarray and mass spectrometric analysis.

Statistical analysis

One-way ANOVA and post hoc multiple mean comparisons were performed on untransformed data for tail moment using SPSS v.15.0.

Results

MAA analysis

Spectrophotometric scans of methanolic extracts of field-collected anemones showed mean absorbance increased substantially in the range at which MAAs would be detected (302 nm – 360 nm) (Fig. 2). Chromatograms from methanolic extracts of anemones indicated the presence of UV-absorbing compounds (Fig. 3). Further investigation of extracts via photodiode array and mass spectrometry revealed measurable quantities of up to 4 MAAs: mycosporine-2-glycine; mycosporine-glycine which co-eluted with shinorine; Porphyra-334 and traces of 2 additional MAAs: usujirene / palythene and palythine.

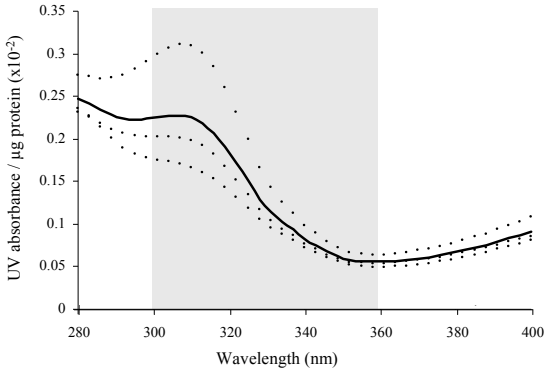

Figure 2: Spectrophotometric scan of 100% methanolic extracts from field-collected anemones. The shaded area represents the range at which MAAs absorb UVR. The solid black line represents the mean absorbance, dotted lines represent the scans of three individual anemones.

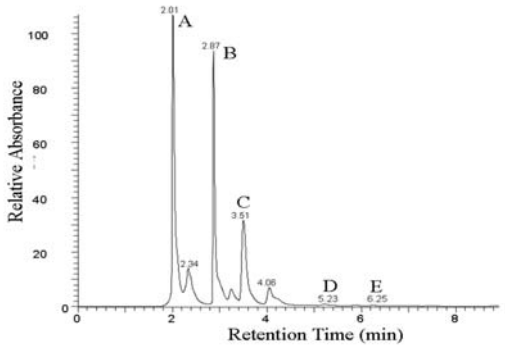

Figure 3: Chromatogram of methanolic extract from *A. pallida* Peak identifications: A - mycosporine-2-glycine; B_1 and B_2 - mycosporine-glycine which co-eluted with shinorine; C – Porphyra-334; D - usujirene / palythene and E – palythine.

Recovery Response

A one-way ANOVA indicated that DNA SB measured as tail moment varies significantly with recovery time ($p = 0.009$). Post Hoc multiple comparisons (Tukey HSD) revealed significant differences between repair times (Fig. 4).

While not statistically significant, tail moment increased by 127% in anemones which received 0 h recovery time compared to control organisms. This difference likely results from SB caused by a combination of UVR and oxidative stress, due to the fact that control groups did not have the added stress of UVR.

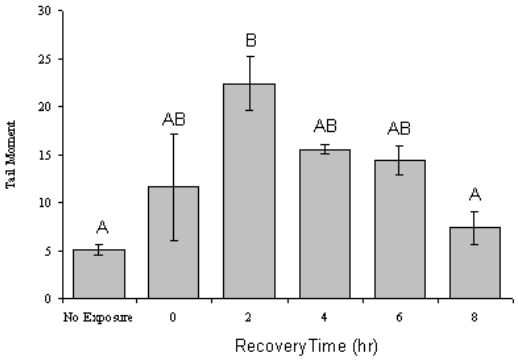

Figure 4: Genotoxic damage and DNA repair evaluated in isolated nuclei from field-collected *A. pallida* using the comet assay. The control group (n=3) received no UV exposure. Bars represent means ±SEM (n=4 anemones with 2 replicate gels per individual). Matching letters indicate non-significant differences at $\alpha = 0.05$

After a two-hour recovery period, tail moment doubled from that at the 0 h recovery time. This lag in time between UVR exposure and the subsequent increased number of breaks in the DNA indicates that excision of photoproducts is well underway at 2 h post-exposure.

The DNA SB at the 4 h recovery time dropped by 29%. However, the decrease in DNA SB from 2 h to 4 h is not statistically significant. This drop in DNA SB is likely attributed to the cells' gradual removal and repair of damage at specific sites of the DNA molecule.

There was not a further significant drop in DNA SB at the 6 h recovery time. However, after 8 h of recovery, tail moment was not significantly different from either controls or pre-exposure levels. This indicates that field-collected animals are capable of repairing DNA damage to a level equivalent to pre-exposure conditions within the course of a night.

Discussion

Spectrophotometric scans of extracts from field-collected anemones indicated peak absorbance at 310 nm, which implies the presence of relatively large quantities of mycosporine-glycine (λ_{max} = 310 nm). Further investigation of these compounds via LC/MS confirmed initial scans and identified measurable quantities of 2 MAAs: mycosporine-2-glycine and mycosporine-glycine, with smaller amounts of shinorine and Porphyra-334. Traces of usujirene / palythene and palythine were also found. Similar to our findings, Stochaj (1989) reported the presence of mycosporine-2-glycine, mycosporine-glycine and shinorine in *A. pallida* from Bermuda but did not find Porphyra-334. Banaszak et al. (1998) also reported the presence of only mycosporine-glycine and shinorine in *A. pallida* from Belize.

The production of MAAs as a defense mechanism against UV damage is well-studied in symbiotic marine cnidarians (Dunlap and Chalker 1986, Drollet et al. 1997, Banaszak et al. 1998). In a recent study, Torregiani and Lesser (2007) observed that corals taken from a depth of 1m had very low concentrations of MAAs and high CPD accumulation.

A defining factor for coral survival depends on the efficiency with which they repair DNA damage. Lesser and Farrell (2004) reported that host DNA damage in the coral *Montastraea faveolata* is intensified under high solar irradiance and thermal stress due to higher oxidative stress and CPD formation. Therefore, the ability to repair such damage is vital. Our investigation shows that field-collected *A. pallida* also exhibit an increase in DNA SB post-UVR exposure (Fig. 4). This is likely, in part, due to base and nucleotide excision repair. The literature regarding UV-induced DNA SB and repair in cnidarians is extremely limited; only one other study of this nature has been reported (Rinkevich et al. 2005). Similar to our investigation, Rinkevich et al. employed the comet assay to demonstrate an increase of DNA SB in a branching coral following a 1 h recovery period immediately after UVR exposure. This increase in DNA SB was attributed to BER and NER. However, a single repair time of 1 h is not sufficient to follow the time course of repair. The repair experiment in our investigation explores multiple recovery periods which allows for a better understanding of the course of DNA repair.

While future UVB levels are difficult to predict, current estimates suggest that it will take at least half a century before UVB levels return to pre-ozone depletion levels. Moreover, for the next century the ozone layer will be at its most vulnerable (Madronich et al. 1998). Our research offers a technique which is easily employed in field-collected samples and could be used to identify increasing DNA damage in stressed corals. This assay will better equip scientists trying to monitor the health of corals, predict bleaching events, or further study the effects of UVR exposure and elevated sea temperatures.

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Induced bleaching of *Stylophora pistillata* by darkness stress and its subsequent recovery

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Abstract. Bleaching, the visible effect of the loss of zooxanthellae by corals has been observed in the last few decades with increasing frequency in most of the world's reefs. Since it usually leads to coral colony death and whole reef destruction, it has been the subject of several studies in recent years. In the present article we describe a method for causing complete, but reversible, bleaching in the common Red Sea coral, *Stylophora pistillata* by darkness stress. This treatment resulted in the reduction of the density of the zooxanthellae, the endosymbiotic algae living within the coral's cells. After 8 days in the dark the coral began to show visible bleaching, which a week later progressed to 70%, and by the 44th day we obtained complete bleaching. At that time no zooxanthellae or chlorophyll were detected in the coral tissue. Following the transferal of the corals to darkness, at first the algal cells exhibited a photoacclimative response, accumulating chlorophyll to levels above the initial values. Fragments that were 70% bleached showed full recovery 30 days after having been returned to light. In the course of recovery, cellular chlorophyll gradually decreased to control – and initial concentrations. From the recovery rate we calculated the maximal doubling rate in the log phase of zooxanthellae repopulating *S. pistillata* to be 2.5 days.

Key words: Coral, bleaching, recovery, chlorophyll.

Introduction

Coral reefs are built by hermatypic corals, in which there is a mutualistic symbiotic relationship between the coral host and single celled microalgae, the zooxanthellae, harbored within the endodermal cells of the coral. The coral affords protection to the algae and provides them with essential nutrients and CO_2 from its metabolic processes. In return, the algae supply the coral with high–energy photosynthate translocated to the animal host. On shallow, well illuminated reefs this nutritional source covers as much as 95% of all metabolic needs of the coral (Falkowski et al 1990). Under high light the energy suffices for maintenance, calcification, growth and reproduction whereas in deep lying, shaded reefs, supplementary predation on zooplankton is needed to satisfy these needs (Dubinsky et al. 1986).

The algae-coral association fine-tunes to ambient light and nutrient regimes by photoacclimation of the zooxanthellae and adjustment of their density, as well as changes in shape and orientation of the entire colony. The photoacclimation is evident from as much as 5 fold changes in the chlorophyll content of the symbionts (Falkowski & Dubinsky 1981; Dubinsky et al. 1984), whereas eutrophication brings about proliferation of the zooxanthellae and reduction of their contribution to the coral (Dubinsky et al. 1990). In branching species the response of the holobiont to high light results in profuse growth in all directions, whereas in the shade or dim light it fans out growing horizontally (Fricke and Schuhmacher 1983). The constant density of symbionts is kept in equilibrium with the growth rate of the colony, as the balance between their division rates, colony expansion and expulsion of excess cells is maintained. It has been found that hermatypic corals release about 0.1%-1% of the algal cells to the water every day, which is usually less then the 0.5%-10% of algae produced in the coral tissue during the same time (Stimson and Kinzie 1991; Hoegh-Guldberg 1994; Titlyanov 1996). In a few cases there are reports of digestion of some of the zooxanthellae by corals (Fabricius et al. 1996). These finely tuned equilibria are maintained on one hand by the translocation of most photoassimilated carbon to the host coral, and on the other hand by the paucity of the key nutrients, nitrogen and phosphorus needed for new zooxanthellar production. A typical doubling rate for the symbionts of *S. pistillata* in nature was 70 and 100 days for high and low light corals, respectively (Falkowski et al 1984).

This equilibrium can be upset if the coral is under stress; if the stress is strong enough the process of bleaching can be triggered, in which case the coral becomes colorless as the white color of the skeleton shows through the thin and transparent tissue (Hoegh-

Guldberg & Smith 1989). The loss of color can occur in two ways: **The coral losing its algae (bleaching);** it has been found that under heat stress the zooxanthellae are expelled from the coral tissue at rates about 1000 higher than in normal conditions (Hoegh-Guldberg & Smith 1989; Ruth et al. 1992). This phenomenon has been described by Glynn (1983) from direct microscopic observation. **The algal cells losing chlorophyll pigments (paling**) – when exposed to a combined heat and high light stress for 7 hours a reduction of ~x 7 in cellular chlorophyll levels has been measured (Salih et al.1998). Chlorophyll content per area of bleached *Agaricia tenuifolia* has been found to be 50 times less than normal (Lovelock et al. 1996).

The converse process, namely the out-of-control proliferation of the zooxanthellae, takes place whenever corals are exposed to anthropogenic (Dubinsky et al 1990) or rarely natural (Genin et al. 1995) eutrophication. This phenomenon, whereby zooxanthellar density increased fivefold in two weeks is detrimental to the colony, since under such conditions nutrients are used up by the algae in their multiplication severely curtailing the life-supporting translocation of photosynthate to the animal (Dubinsky and Jokiel 1994; Dubinsky and Berman-Frank 2001).

Bleaching events stop skeleton growth of the coral (Glynn 1983; Glynn and D'Croz 1990) and usually cause colony death and entire reef collapse (Brown & Suharsono 1990).

The chances of recovery following bleaching depend on the severity of the causative stress, its nature, intensity and duration, and on the conditions after the event. It has been suggested that in some cases it is possible that bleaching and subsequent recovery are the coral's way to achieve quick genetic change in the zooxanthellar community to populations better suited to withstand stress and react to environmental change (Buddemeier & Fautin 1993).

The recovery process can be achieved in two ways: fast reproduction of the "best" algal clone within the coral or by acquisition of a new clone from the water in the immediate environment (Goreau 1991; Rowan and Knowlton 1995).

Stylophora pistillata is the most abundant reef building coral in the Gulf of Eilat (Northern Red Sea) in both numbers and area coverage (Loya 1976). It is an "r" strategist (Loya 1972) which outcompetes all other species in the struggle to monopolize areas becoming available following natural or man-made disturbances. Since it also easily recovers from experimental handling and breakage into fragments it has been a favored model organism in the study of coral biology (e.g. Loya 2000).

Darkness Stress

Due to its dependence on the photosynthesis of the algal symbionts, darkness is a powerful stressor of zooxanthellate corals (Rogers 1979). By placing the coral in a light-tight container that blocks all light without affecting any other parameter such as temperature, pH or flow regime we easily cause darkness stress. Goreau (1959) found that when *Manicina areolata* was maintained in darkness for 10 days to two months it bleached.

Bleaching in darkness is caused by the loss of zooxanthellar cells, as found by Kevin & Hudson (1979) in a study of the cold water coral *Plesiastrea urvillei*.

Materials and Methods

Three colonies of *Stylophora pistillata* were collected from the reef near the Interuniversity Institute, Gulf of Eilat (Aqaba), Israel. Corals of 15-cm diameter were collected from artificial objects at a depth of 2.5-3.5 m. The corals were allowed to acclimate for one month in our main aquarium system under irradiance of 500 µmol quanta $m^{-2}s^{-1}$. Then the corals were divided into 3-cm-long fragments and glued with Super Glue Gel (Scotch, 3M) to plastic tips (Fig. 1).

Figure 1: *Stylophora pistillata* fragments during acclimation in our main aquarium system.

The fragments were left undisturbed for a period of 4 weeks for recovery and acclimation. Only fragments that showed a beginning of tissue growth (onto the plastic tip) were taken for the experiment.
The experiment was performed in a closed system at Bar-Ilan University.

Lighting was obtained from 3 fluorescent lamps of type T5 (10,000K, ATI), which delivered 500µmol quanta $m^{-2}s^{-1}$ at the water surface.

The Darkness Experiment

The experiment was conducted in two plastic containers, 10 L each, with openings for water exchange. The openings were connected by a "T" fitting preventing light from penetrating. A black plastic sheet assuring darkness covered one container and the other container was left uncovered as the control. The two containers were connected to the main water supply system. The light regime of the system was a 12 hours light and 12 hours dark photoperiod.

In each container were placed 30 fragments (a total of 60 fragments). Once every two weeks, 3 fragments from each container were removed and the following were performed: Determining algal areal density and measuring chlorophyll concentration per area and cell. Sixteen days after the beginning of the darkness treatment fragments showed 70% bleaching. At that time 9 fragments from the darkness treatment were transferred for recovery to the illuminated control tank. We monitored their recovery process over six weeks, by taking the same measurements described above for three fragments every two weeks.

We used standard methods for quantifying chlorophyll density in *Stylophora pistillata* and other coral species (Dubinsky et al. 1990; Titlyanov et al. 2000; Nordemar et al. 2003). The procedure requires sacrificing the sample using the Water-Pik method (Johannes and Wiebe 1970; Falkowski and Dubinsky 1981; Hoegh-Guldberg and Smith 1989; Edmunds and Gates 2002) to remove the live animal tissue with the zooxanthellae from the coral skeleton. The resulting homogenate was filtered through a 25-mm Ø glass fiber paper filter (Whatman GFC). The zooxanthellae collected on the filter were extracted in 10 ml of 90% acetone/water by grinding the filter with a glass/PTF homogenizer. The resulting slurry was filtered again through the same type of filter. Chlorophyll concentration was quantified by measuring the optical absorption at 665 nm and 755 nm with a Varian DMS 100 spectrophotometer according to the Jeffrey and Humphrey (1975) equations, and normalized to sample area.

The fragment area was determined using the standard aluminum foil weight method (Falkowski and Dubinsky 1981; Hoegh-Guldberg and Smith 1989; Edmunds and Gates 2002).

Results & Discussion

The difference between the control and the dark treatment was statistically significant, as already by the 7th day areal chlorophyll decreased by 70% (t-test, n=3, $p<0.05$). By the 44th day when the experiment was terminated, the dark treated fragments had totally bleached, and looked completely white; as the zooxanthellae were lost, the coral tissue became transparent and the white skeleton was revealed (Figure 2).

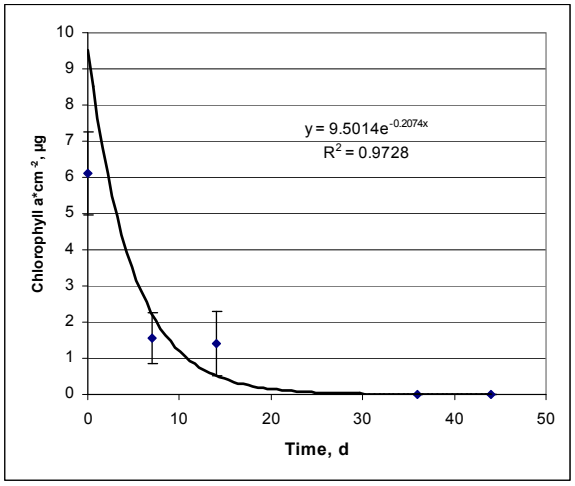

Figure 2: *Areal chlorophyll of bleached* S. pistillata *fragments in darkness (n=3).*

From the plot of chl *a* per zooxanthella cell (figure 3) we can learn that in the first 30 days the dominant factor in the bleaching in darkness was the loss of zooxanthellae, whose numbers began declining immediately; by the end of the experiment less than 1% of the symbionts remained.

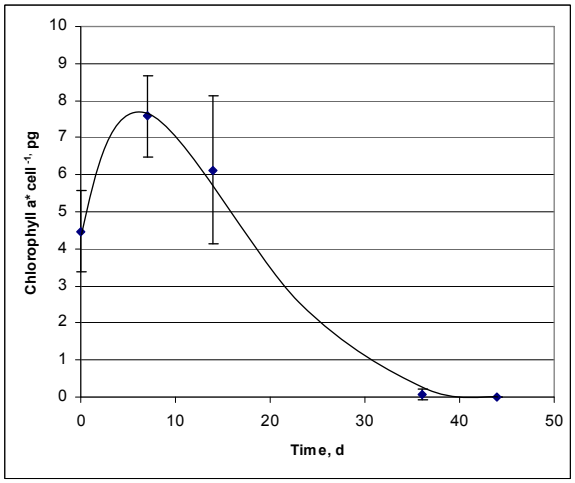

Figure 3: *Chlorophyll per zooxanthella cell of bleached* S. pistillata *fragments (n=3).*

The plot of chlorophyll *a* per zooxanthella (figure 3) shows that during the first 10 days after the corals were placed in full darkness the zooxanthellae were actually gaining chlorophyll, which is in agreement with the photoacclimation studies on *S. pistillata* (Falkowski & Dubinsky 1981; Dubinsky et al. 1983).

The recovery of the coral's chlorophyll concentration is shown in figure 4.

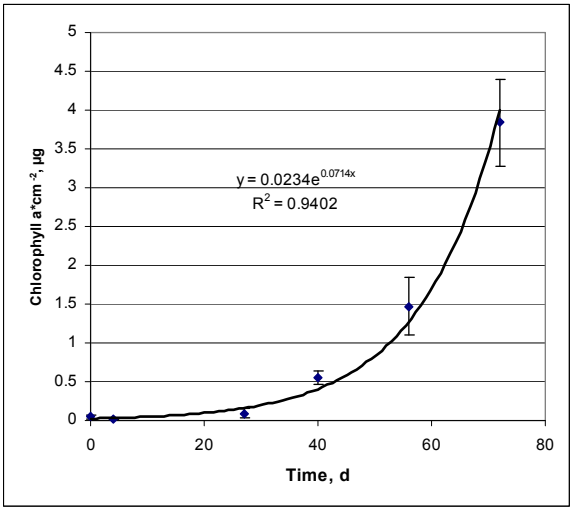

Figure 4: *Chlorophyll concentration in* S. pistillata *fragments recovering from darkness bleaching*

In the first 20 days the chlorophyll concentration in the coral tissue did not change much. In the plot following the changes in the chlorophyll in the zooxanthella cells (figure 5) we can see that in the first 10 days the chlorophyll content per cell was decreasing. We suggest that again it could be explained as a photoacclimation of the zooxanthellae upon being suddenly transferred from darkness to bright light.

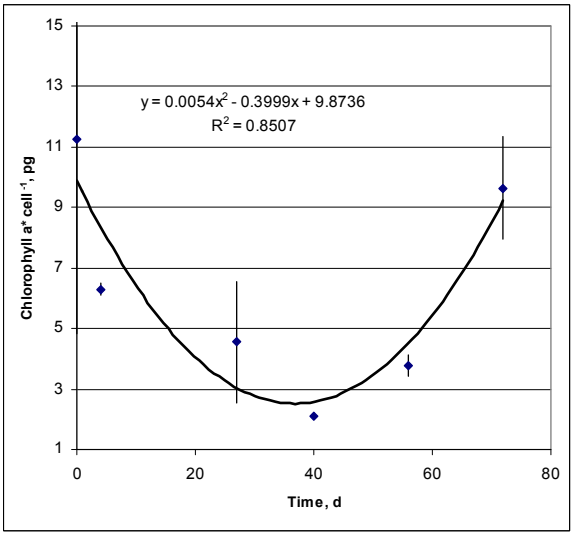

Figure 5: *Chlorophyll per zooxanthella in* S. pistillata *fragments recovering from darkness.*

After 20 days the chlorophyll and the algal population were being restored rapidly, until they reached initial and control levels at about the 70th day.

We followed the increase in zooxanthella density per cm^2 and calculated their rate of doubling and found that the fastest doubling time (after the initial lag period) was about 2.5 days (figure 6).

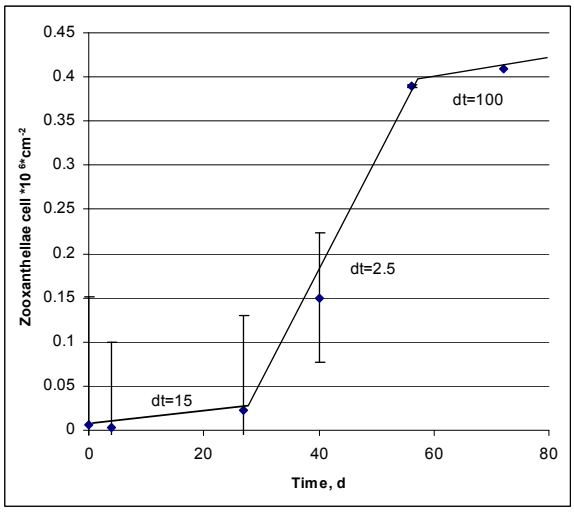

Figure 6: *Zooxanthella doubling rate in* S. pistillata *fragments recovering from darkness*

That is probably the maximal potential doubling rate of the zooxanthellae in the coral tissue when they are not constrained by their own population. Doubling times in steady state (presumably at their carrying capacity in the tissue) were determined as 70 and 100 days in high and low light *S. pistillata* colonies, respectively (Falkowski et al 1984).

Conclusions

The study showed that in the common Red Sea coral *S. pistillata* darkness leads to bleaching. However, presumably a few zooxanthella cells remained, and upon illumination, these rapidly repopulated the bleached coral tissue. It is especially noteworthy that the bleached corals survived in total darkness for nearly two months, and that the doubling rate of the zooxanthellae during the recovery phase was as short as 2.5 days, whereas in nature it was reported to be 70-100 days. We interpret it as representing a population's reaction to finding itself way below its carrying capacity, thereby realizing its maximal potential growth rate.

On the technical level the study illustrates the power of the described photographic method as a tool in coral research.

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Thermal stress increases oxidative DNA damage in coral cell aggregates

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Abstract. We have established an experimental system to study the response of coral cells to stresses using coral cell aggregates (tissue balls). The objective of this study was to evaluate the use of tissue balls for studies of oxidative DNA damage in corals cells under thermal stress. Tissue balls prepared from dissociated cells of *Pavona divaricata* were exposed to high temperature (31°C) in the presence or absence of an antioxidant or to normal temperature (25°C). DNA damage of coral cells was investigated using a Comet Assay (alkaline single-cell gel electrophoresis). The comet tail length, which indicates the degree of DNA damage, was significantly longer in coral cells from tissue balls exposed to 31°C than those from the controls (25°C) or those treated at 31°C in the presence of an antioxidant, 10 mM mannitol. The present results suggest that coral cells suffer oxidative DNA damage under thermal stress. It was not clear whether DNA damage occurred in algal cells as algal DNA was retained within the cell wall and the comet tail was not observed. This study also showed that tissue balls provide us with a good experimental system to study the effect of stress and various chemical reagents on coral cells.

Key words: Coral, Comet assay, DNA damage, Bleaching

Introduction

Coral reefs are under threats due to increasing sea surface temperature (SST) that cause coral bleaching. A number of laboratory and field studies have reported the link between high temperature stress and coral bleaching (Hoegh-Guldberg and Smith 1989; Fitt et al 1993; Lesser 1997; Hoegh-Guldberg 1999). High temperature stress has been proposed to produce damage of the algal photosynthetic apparatus, which leads to production of reactive oxygen species (ROS) (eLesser 1997; Jones et al. 1998; Bhagooli and Hidaka 2004; Yakovleva and Hidaka 2004) Although cells have an enzymatic antioxidant system to scavenge the harmful ROS (Downs et al. 2002; Lesser and Farrell 2004), the excess ROS cause damage in cellular components such as protein carbonilation (Downs et al. 2002; Richier et al. 2005), lipid peroxidation (Richier et al. 2005) and DNA degradation (Lesser and Farrell 2004). Recent studies showed that apoptotic or necrotic death of host cells are involved in cnidarian bleaching (Lesser and Farrell 2004; Richier et al. 2006; Dunn et al. 2007). On the other hand, zooxanthellae expelled by coral hosts during bleaching exhibit cell degeneration and apoptotic or necrotic death (Franklin et al. 2004; Strychar et al. 2004). However, until now the direct information about the effect of thermal stress on the DNA level in reef-building corals is very limited.

We have established an experimental system to study the response of coral cells to stress treatment using coral cell aggregates (tissue balls) (Nesa and Hidaka, submitted). Dissociated coral cells aggregate to form spherical bodies, which rotate by ciliary movement. These spherical bodies (tissue balls) stop their rotation and become disintegrated when exposed to stress. The relationship between the survival time and zooxanthella density of tissue balls showed a negative correlation at 31°C, while no significant correlation between the survival time and zooxanthella density of tissue balls was found at 25°C (Nesa and Hidaka, 2009). The results support the hypothesis that zooxanthellae become a burden for host corals under thermal stress probably via production of harmful substances such as reactive oxygen species (ROS).

In this study the use of tissue balls for the study of oxidative DNA damage of coral and algal cells under thermal stress was evaluated. We used the comet assay (single cell gel electrophoresis) to detect DNA damage in individual target cells (e.g., Avishai et al. 2003; Rinkevich et al. 2005).

Material and Methods

Collection and Maintenance of coral specimens

Small colonies of *Pavona divaricata* were collected from the reef at Bise, northern Okinawa. Corals were then brought to the Nishihara campus of the University of the Ryukyus and were maintained in an aquarium provided with a subgravel filter at 26°C under 150 µmol quanta $m^{-2}s^{-1}$ on a 12:12h light/dark cycle for up to 2 weeks prior to use.

Preparation of tissue balls

Pavona divaricata fragments were rinsed gently with 0.22 µm filtered seawater (FSW) and then coral tissue was removed from the skeleton using a WaterPik (Teledyne, WP-70J). To remove the mucus produced during the isolation procedure, coral slurry was filtered through 180 µm nylon mesh. The slurry was then homogenized with a homogenizer and filtration through 40 µm nylon mesh was performed. The resulting homogenate was centrifuged at 1200 rpm for 5 min, and then at 1000 rpm for 5 min. The supernatant was discarded and the pellet was re-suspended in FSW using a vortexer. The suspension containing dissociated coral cells and zooxanthellae was incubated in a 24-well plate. In each well 1 ml of dissociated coral-zooxanthella cell suspension was incubated with 1 ml 0.22 µm FSW for one night at room temperature. After overnight incubation, dissociated coral cells and zooxanthellae became aggregated and achieved a spherical shape. These spherical bodies (tissue balls) started to rotate by ciliary movement. After one night tissue balls were transferred to a clean petridish with fresh FSW. Healthy (rotating) tissue balls of similar size were chosen under a stereomicroscope (Nikon-SMZ-10) and put separately in each well of a 96-well plate containing 280 µl FSW. These preparations were allowed to recover at room temperature for 3-6 hours before the stress experiments. Although tissue balls contained various numbers of zooxanthellae, the density of zooxanthellae in the tissue balls was not determined in this study.

Treatments of tissue balls

In the first series of experiments, tissue balls were exposed to normal (25°C) or high (31°C) temperature in filtered seawater (FSW) for 10 h. Two 96-well plates, each with 25 tissue balls, were prepared and one plate was placed in an incubator (Yamato, Program Incubator IQ820) set at 25°C and the other plate was placed in another incubator (Sanyo, MIR-152) set at 31°C. In both incubators, the plates were illuminated using a 19W fluorescent light and the light intensity measured by a light meter (LICOR, LI-250) was 35 µmol quanta m^{-2} s^{-1}. In the second series of experiments, tissue balls were exposed to normal (25°C) temperature in FSW or high (31°C) temperature in FSW containing 10 mM mannitol, a scavenger of hydroxyl radicals. In both series of experiments, the experiment was repeated 3 times.

The comet assay

To detect possible DNA damage in coral cells, we used a Comet Assay kit (Trevigen). In the comet assay, cells were embedded in agarose on a microscopic slide. Cells were lysed to remove cytoplasm and most nuclear protein, leaving supercoiled DNA as 'nucleoids'. During electrophoresis DNA is attracted to the anode, but if breaks are present, a "comet tail' of DNA extending from the nucleoid is seen. The size and shape of the comet reflects the extent of DNA damage.

After exposure to thermal stress, tissue balls were placed into 1 ml of ice cold 0.01M PBS containing 20 mM EDTA and dissociated into single cells by pipetting for two minutes. Cells were collected by centrifugation (1,500 g, 1 min) and re-suspended in 300 µl of 0.01 M PBS containing 20 mM EDTA. 50 µl of the cell suspension was mixed with 500 µl low melting agarose (LMA). 75 µl of this mixture was added onto a comet slide and homogeneously spread using a pipette tip. The slides were placed in a refrigerator (4°C) for 20 min. For coral cell lysis, slides were then immersed in a lysis solution containing 1% sodium lauryl sarcosinate (provided with the kit) for 30 min at 4°C. Slides were immersed in alkaline solution containing 200 mM EDTA (pH 13) at room temperature for 20 minutes. Slides were then placed on a horizontal electrophoresis apparatus. TBE electrophoresis buffer was added to the electrophoresis tray to cover the slides. Electrophoresis was conducted at 20-22 volts and 0.01 mA for 10 min at room temperature. After electrophoresis, slides were immersed in 70% EtOH for 5 min. DNA staining was performed by adding 50 µl SYBR Green I (SYBR Green I 10 µl + 1 X TE buffer 200µl) to each circle of slides. All steps were performed under dimmed light to prevent additional DNA damage during the procedure.

For zooxanthella cell lysis, we used a modified Comet Assay following Erbes et al. (1997). Slides were immersed in alkaline lysing solution containing ionic detergent (0.1% Sodium Dodecyl Sulfate) for 10 min at room temperature. Slides were immersed in alkaline electrophoresis solution for 5 min at 4°C. Slides were then placed on a horizontal electrophoresis apparatus. Alkaline electrophoresis solution was added to the electrophoresis tray to cover the slides. Electrophoresis was conducted at 20-22 volts and 0.01 mA for 10 min at 4°C. After

electrophoresis, slides were immersed in Tris buffer for 5 min.

Image analysis

Samples were visualized and photographed on a fluorescence microscope (Nikon OPTIPHOT-2) at 20 X or 40 X using a digital camera (Nikon Digital Sight DS-LI). Comet tail lengths were measured by using Image J (1.40) software. At least 50 comets were measured in each condition in each of three replicated experiments. Comet tail length is easy to measure and still is a good parameter of DNA damage in sample cells (reviewed by Lee and Steinert 2003).

Statistical analysis

The experiment was repeated three times and the average comet tail length in each condition was used as the statistical unit. A statistical program, Stat view for windows (5.0.1) was used for data analysis. A Mann Whitney U-test was performed to test the significant differences of the comet tail length among the treatment groups. A p value <0.05 was considered to be significant.

Results

DNA damage in coral cells

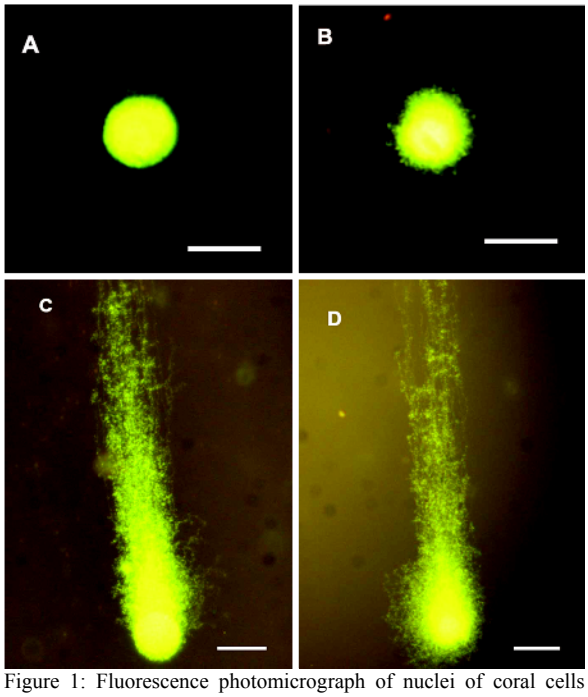

Figure 1: Fluorescence photomicrograph of nuclei of coral cells after alkaline single cell electrophoresis (Comet Assay). A and B, nuclei of coral cells from the control tissue balls kept at 25°C. C and D, nuclei of coral cells from tissue balls exposed to high temperature (31°C). Scale bar = 10 µm

The nuclei of coral cells from the control tissue balls (25°C) was circular without a tail (Fig. 1A) or with a very small tail (Fig. 1B), while the nuclei of cells from the tissue balls exposed to high temperature (31°C) showed comets with a long tail (Fig. 1C, D).

In this study, we measured comet tail length as an indicator of DNA damage of cells within the tissue balls exposed to different stress conditions. The comet tail length of high temperature-treated coral cells was significantly longer (Fig. 2) than that of the control cells (Mann Whitney U test, $p < 0.05$).

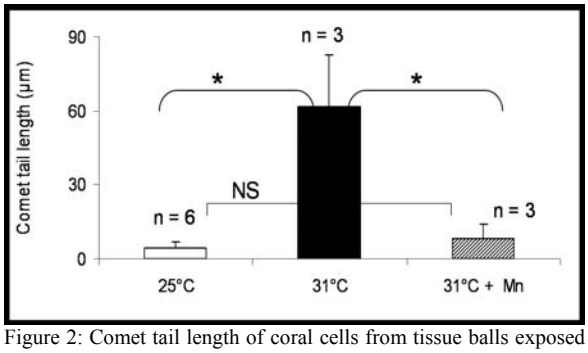

Figure 2: Comet tail length of coral cells from tissue balls exposed to different treatment conditions; open column represents the control (25°C), closed column represents high temperature (31°C) treatment and striped column represents high temperature (31°C) treatment in the presence of 10 mM mannitol.

The coral cells from tissue balls exposed to high temperature (31°C) in the presence of 10 mM mannitol showed significantly shorter comet tail length (Fig. 2) than those from tissue balls exposed to high temperature in the absence of mannitol (Mann Whitney U test, $p < 0.05$). There was no significant difference in the comet tail length between the cells from tissue balls exposed to high temperature in the presence of mannitol and those from the control tissue balls. The comet tail length was 4.3 ± 2.8 µm (n=6), 61.7 ± 21.2 µm (n=3), and 8.0 ± 6.0 µm (n=3) for the control, high temperature-treated tissue balls, and high temperature-treated tissue balls in the presence of the antioxidant, respectively.

DNA damage in algal cells:

The analyzed images of comet assays for zooxanthellae using alkaline lysing solution containing ionic detergent revealed that most of the zooxanthellae from both control and HT treatments showed a distinct head without a tail, or with a small tail (Fig. 3). The comet tail length of algal cells was 0.14 ± 0.19 µm (n=3) and 0.36 ± 0.18 µm (n=3), for the controls (25°C) and high temperature (31°C) treated tissue balls, respectively. No significant difference was found in the length of comet tails of zooxanthellae between the control and HT treatment (U-test, $p=0.275$).

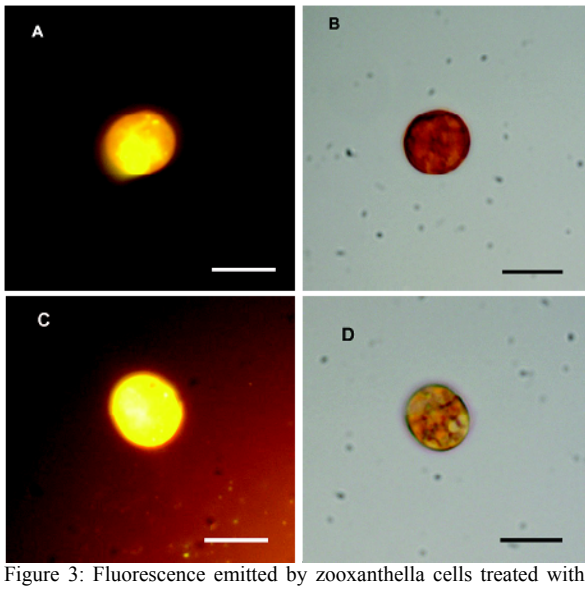

Figure 3: Fluorescence emitted by zooxanthella cells treated with different conditions (A, C); A and B represent control (25°C), and C and D represent high temperature (31°C) treatment. B and D are light microscopy images. Scale bar = 10 µm

Discussion

The present results clearly showed that coral cells suffer DNA damage under thermal stress. The addition of an antioxidant, mannitol, decreased the extent of DNA damage in tissue balls exposed to high temperature (31°C). This shows that tissue balls suffer oxidative DNA damage probably due to ROS produced by symbiotic algae under thermal stress. Addition of mannitol extended the survival time of *Pavona divaricata* tissue balls suggesting that this antioxidant is effective in protecting cellular integrity of corals (Nesa and Hidaka, 2009).

Apoptotic death of host cells (Richier et al. 2006; Dunn et al. 2007) or algal cells (Strychar et al. 2004) might be involved in cnidarian bleaching. While apoptosis results in DNA fragmentation, DNA damage may lead to apoptosis. Rinkevich et al. (2005) reported that UVB irradiation increases DNA breaks of coral and algal cells. It is likely that the DNA damage detected in the present study is due to direct action of ROS produced by symbiotic algae under thermal stress. Further study using inhibitors or inducers of apoptosis and other cell death pathways may be helpful to understand the death pathway of host and algal cells under thermal stress. The present results also suggest that tissue balls provide us with a useful experimental system for studies of cell death under stress conditions.

In the present study, nuclei of algal cells from tissue balls exposed to high temperature did not show a typical comet shape with a long tail. It is contrary to our expectations as symbiotic algae might be a source of ROS (Nesa and Hidaka, 2009). If ROS are produced in the chloroplast of the symbiotic algae, algal DNA is most likely to suffer oxidative damage. Rinkevich et al. (2005) detected DNA damage in isolated algal cells caused by UVB irradiation using the Comet Assay. It is not clear whether algal DNA did not suffer oxidative damage in our experiments, or DNA was actually fragmented but fragmented DNA was confined within a rigid cell wall. The modified Comet Assay, which uses an alkaline lysis solution, was applicable to the single-cell alga *Chlamydomonas*. But it is still to be confirmed whether the modified Comet Assay is applicable to *Symbiodinium* spp. Further studies using a transmission electron microscope might be helpful to understand the damage in algal cells under thermal stress.

Rinkevich et al. (2005) found that the holobiont (isolated coral cells containing symbiotic algae) is more sensitive to UVB radiation than isolated coral cells without symbionts or isolated algal cells. Their finding that symbiotic cnidarians are highly sensitive to ROS produced by UVB is consistent with other recent studies; tissue balls with higher densities of zooxanthellae had shorter survival times than those with lower densities of zooxanthellae under thermal stress (Nesa and Hidaka, 2009), and coral larvae with zooxanthellae had lower survivorship and higher levels of oxidative cellular damage than larvae of the same species that lack zooxanthellae (Yakovleva et al. 2009). Experimental systems using planula larvae, tissue balls, and isolated cells have their own advantages and disadvantages, but they might provide useful information on the role of symbionts in cellular damage of the holobiont under stress conditions.

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Feeding corals in captivity: uptake of four *Artemia*-based feeds by *Galaxea fascicularis*.

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This study evaluates the capture efficiency of the scleractinian coral *Galaxea fascicularis* for four *Artemia*-based feeds: (1) live, non-enriched *Artemia* nauplii; (2) Instant Baby Brine Shrimp (IBBS, pasteurized *Artemia* nauplii); (3) live, SELCO-enriched *Artemia* nauplii, and (4) live *Artemia* nauplii enriched with SELCO that was supplemented with the antibiotics Sulfamethoxazole and Trimethoprim. All prey types were rapidly consumed by the corals (6-11 nauplii per polyp per hour), showing that (I), IBBS can be a cost-effective alternative to feeding freshly hatched nauplii, and (II), corals can be provided with specific additives via their food. The corals preferred live *Artemia* over IBBS, indicating that IBBS can be further optimised for use in coral reef aquaria. SELCO-enriched *Artemia* and medicated *Artemia* were also consumed at lower rates (40% and 30% lower, respectively) than non-enriched *Artemia*. However, these results are a useful first step towards (I), development of coral-specific feed-enrichments, and (II), oral treatment of diseased corals in captivity.

Key words: *Galaxea fascicularis*, feeding, *Artemia* nauplii

Introduction

The keeping of scleractinian (stony) corals in aquaria has become increasingly popular over the last few decades (Wabnitz et al. 2003). Numerous innovations in aquarium technology and improved methods for coral husbandry have made it relatively easy to keep captive corals alive (see overviews by Borneman 2001; Delbeek and Sprung 2005). The present study deals with two topics in coral husbandry that up till now have received relatively little attention: heterotrophic feeding and treatment of diseases.

Scleractinian corals are known to possess two major mechanisms for fulfilling their nutritional needs; photosynthesis through symbiotic zooxanthellae and heterotrophy. They ingest a wide variety of food, such as dissolved and particulate organic matter, sediment, bacteria and zooplankton (Sorokin 1973; Titlyanov and Titlyanova 2002). The importance of heterotrophy relative to autotrophy varies with species and habitat. According to Titlyanov and Titlyanova (2002), the relative contribution of photoassimilation to the total nutritional requirements of zooxanthellate corals ranges from 30 to 90%. Under high-light conditions, such as in shallow, tropical waters, the metabolic energy provided by photosynthesis alone exceeds the coral's daily energetic needs (Edmunds and Davies 1986 and references therein). Deep living corals, or corals living in turbid environments, are more dependent on heterotrophy (Falkowski et al. 1984; Anthony 2000).

Most aquarium corals thrive well in well-illuminated tanks. Therefore, much literature on culturing corals in aquaria focuses on technology for lighting, whereas quantitative studies on heterotrophic nutrition remain scarce. There is consensus among aquarists that at least a minimal amount of food is needed to supplement the resources provided by photosynthesis. Evidence is growing that enhanced feeding can stimulate coral growth considerably (Buongiorni et al. 2003; Ferrier-Pages et al. 2003; Houlbreque et al. 2004), thus emphasizing the importance of improving feeding methods for aquarium corals.

Artemia salina nauplii are widely used for feeding corals in aquaria and are commercially available in the form of dried cysts. Cysts hatch after 18-24 hours and the young nauplii can be fed immediately. Even though hatched nauplii can be

stored up to 48 hours (2-4 °C) without losing any nutritional value (Léger et al. 1983), methods to store them longer, in large quantities and especially without having to hatch them, could be more economically attractive in terms of labour and time management. For this reason, INVE® (Dendermonde, Belgium), a company specialized in food products for aquarium animals, has developed Instant Baby Brine Shrimp (IBBS): dead, pasteurized *Artemia* nauplii that can be stored for one year and fed until 6 weeks after opening. This product would be extremely useful for feeding corals in public and private aquaria. Therefore, the product was tested by comparing the rate at which the scleractinian coral *Galaxea fascicularis* captures IBBS to the rate at which it captures freshly hatched, live *Artemia* nauplii.

A second possibility to improve coral breeding and husbandry is provided by the technology to enrich *Artemia* nauplii with specific components. SELCO (Self Emulsified Lipid Concentrate) is one of the most commonly used enrichments in aquaculture. Specific components such as pro-biotics, antibiotics and food supplements can be coupled to SELCO so that they accumulate in the SELCO-fed nauplii. This technology has been successfully applied by Chair et al (1996) to treat diseases in aquacultured fish and shrimp by using *Artemia* nauplii as a vector for oral addition of antibiotics. This principle may also be applied to corals. During the last decades, extensive research has been done on coral diseases, as large outbreaks seem to become more common on reefs in the wild (Richardson 1998; Weil et al. 2006). Moreover, a disease outbreak can be disastrous in an aquarium. Recently, pathogens have been identified that cause, or are at least associated with coral diseases (Luna et al. 2007; Rosenberg et al. 2007). These findings will enable targeted treatment of coral diseases using specific antibiotics. In this study, a first step is made towards development of treatments of coral diseases through oral delivery of antibiotics: it was tested whether corals capture medicated *Artemia* nauplii. The uptake efficiency of eight colonies of *Galaxea fascicularis* was compared to their uptake efficiency for non-enriched nauplii.

Materials and Methods

Coral materials

Experiments were carried out in the laboratory of the aquarium in Burgers' Zoo, Arnhem, The Netherlands. The coral species used, *Galaxea fascicularis*, was grown in the coral nursery of Burgers' Zoo, registered under number ARKS 611377. One mother colony was fragmented into nine smaller colonies, which were attached to small PVC plates of 5 X 5 cm. Hence, all colonies were genetically identical. Eight colonies of 8 x 8 cm were used for the experiment. The number of polyps of each colony were counted as an estimate for coral biomass.

Methods

Feeding experiments were conducted in cylindrical Perspex incubation chambers with a volume of 1.4 l. The water used was taken directly from the tank in which the corals were kept in between experiments, to reduce ambient stress for the corals as much as possible. During experiments, conditions in the incubation chambers were kept the same as in the tank (Table 1). Water temperature was controlled by a TECO TC20 water cooler (TECO, Ravenna, Italy) and kept constant at 26°C. Light was provided by a 70 Watt HQI lamp, type BLV, 10.000 Kelvin. Water flow was controlled by an IKA® Big-Squid Ocean magnetic stirring plate and kept constant at 2.4 notches; at this speed (resulting in a water velocity of approximately 10 cm/s), the *Artemia* nauplii remained in suspension. Colonies were incubated one at a time for measurements of capture rates.

Table 1: Water conditions in the maintenance tank and in the incubation chamber.

Temperature	26°C
Salinity	34 ppt
Calcium	400-410 mg/l
Alkalinity	2.5-3.0 mEq/l
Nitrate	< 0.02 mg NO_3-N/l
Phosphate	< 0.01 mg PO_4/l

All feeding experiments were carried out by adding an initial concentration of approximately 2000 nauplii / l. Colonies were allowed to feed for 15 minutes. A pilot study had revealed that at these experimental settings, neither saturation of the corals, nor depletion of the food resource occurred, thus making it possible to compare capture rates for different feeds.

Before the start of each experiment, the concentration of nauplii in a concentrated batch was calculated by counting the number of nauplii in 15 replicate samples of 200 µl taken from that batch. From this, the volume to be added to the incubation chamber was calculated and added. Coral colonies were allowed to acclimatise in the respiration chamber for 20 minutes before introduction of the food. A sample of 50 ml was taken from the

respiration chamber with a syringe two minutes after adding the nauplii and the number of nauplii in the sample was counted under a binocular microscope. A second sample was taken 15 minutes after adding the nauplii. Capture rates were expressed as the number of nauplii cleared from the water in the respiration chamber in this 13 minute interval per coral polyp.

Experiments
All colonies were subjected to four feeding treatments:
Treatment 1: Non-enriched nauplii. *Artemia* nauplii from Great Salt Lake (Aquafeed, Utah, USA) were hatched by the Zoo's aquarists at temperatures of 25-26°C and used when 26 hours old.
Treatment 2: Ocean Nutrition Instant Baby Brine Shrimp (IBBS, dead *Artemia* nauplii, manufactured and supplied by INVE® (Dendermonde, Belgium).
Treatment 3: Enriched nauplii. 0.17 ml Easy DHA SELCO enrichment per litre was added once to 30 hour old *Artemia* nauplii. 19 hours after this, the batch was put on air and stored in a refrigerator during the day to ensure that the *Artemia* used at the end of the day had the same SELCO content as the ones used at the start of the day (Léger et al. 1983).
Treatment 4: *Artemia* were enriched with medicated Easy DHA SELCO, consisting of an emulsion with 5% Sulfamethoxazole/Trimethoprim (5:1), prepared by INVE®. Storage was done as described in Treatment 3.
Every treatment was repeated 3 times for each replicate colony (i.e. 24 experiments per treatment) before switching to the next treatment. The colonies were not fed by the aquarium staff on the days of the experiments. All colonies were given at least one day to recuperate from the experimental treatment in the maintenance tank, during which they were fed by the aquarium staff.

Blank controls
For every treatment, 8 blank control tests were conducted to check for the accuracy of the start concentration of added nauplii and for possible effects of the chamber on nauplii concentration. Blank tests were conducted following the protocol of the incubations with corals. A limestone coral skeleton in the shape of those of the experimental colonies was placed in the chamber to create a similar flow pattern.

Carbon content
The carbon content of the live *Artemia*-based products was measured to enable expression of uptake rate in organic carbon equivalents. Enriched *Artemia* are fed as nauplii of the Instar II type, while freshly hatched *Artemia* are fed as the smaller-sized Instar I type. Organic carbon was determined by the Wet Oxidation Method, using an OIC 700 Total Organic Carbon analyzer.

Data analysis
Statistical analysis of the data was done with SPSS 12.01. Data were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Homogeneity of variances was tested by a Levene's Test for Equal Variances. Paired-Samples T-Tests and Mann-Whitney tests were used for determining differences in samples from the blank controls taken at t = 0 and t = 15 minutes, respectively. To determine differences in uptake rates between the different treatments, Paired-Samples T-Tests were used ($\alpha = 0.05$).

Results
It was observed that all coral colonies responded immediately to all treatments by capturing prey from the moment it was added to the chamber. No significant reduction in nauplii numbers was observed in the blank controls, except for IBBS. The IBBS were observed to stick to dead surfaces easily (both in blank controls and in incubations with corals). Due to this stickiness, the numbers of IBBS in the blank controls decreased by 14% within the 13 minute experimental interval. This was taken into account by subtracting this difference from the number of consumed IBBS in the experiments with corals.

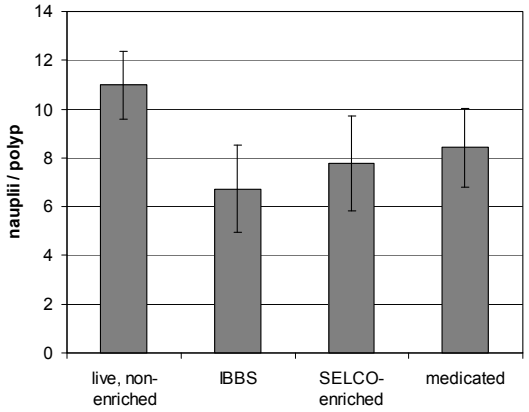

Figure 1. Average number of nauplii captured per polyp within the 13 minute incubation interval. Error bars indicate standard deviations (n = 24).

The average capture rates (expressed as nauplii captured per polyp within the 13 minute time

interval) for the different prey types were 10.98 (freshly hatched), 6.73 (IBBS), 7.77 (SELCO-enriched) and 8.42 (medicated) (Fig. 1). The uptake of freshly hatched, non-enriched nauplii was significantly higher than the uptake of the other prey types ($p < 0.001$). The reduction in capture rate was 38% for LBBS, 29% for SELCO-enriched nauplii and 23% for SELCO-enriched nauplii supplemented with antibiotics.

When normalized to organic carbon (Table 2), uptake per polyp was highest for medicated *Artemia*, which had a carbon content that was slightly higher than that of freshly hatched, non-enriched nauplii. Surprisingly, SELCO-enriched Instar II nauplii had a lower carbon content than freshly hatched, non-enriched nauplii. The measured carbon contents were comparable to literature data: Ohman (1987) reported a value of 0.5 µg C per nauplius, Evjemo and Olsen (1999) reported a value of 0.77-0.95 µg C per nauplius.

Table 2. Organic carbon content per individual for live *Artemia* products and calculated mean organic carbon uptake per polyp within the 13 minute incubation interval.

Treatment	Organic carbon content (µgC)	Organic carbon uptake (µgC/polyp)
Live, non-enriched	0.30	3.29
SELCO-enriched	0.25	1.94
Medicated	0.42	3.54

Discussion

Live Artemia *nauplii versus Instant Baby Brine Shrimp*

Our study showed some limitations of Instant Baby Brine Shrimp for feeding corals in captivity. The scleractinian coral *Galaxea fascicularis* preferred live, non-enriched *Artemia* nauplii over IBBS. Besides, IBBS tend to precipitate and stick to other surfaces. We conducted a similar experiment on another coral species, *Seriatopora caliendrum*, which also significantly preferred freshly hatched live nauplii over IBBS, IBBS uptake rates being on average 80% lower. However, the fact that IBBS are captured by both coral species demonstrates that the product is in principle suitable for coral culture.

In order to improve the IBBS product for feeding corals, it is important to understand why live nauplii are preferred. Live zooplankters are able to swim and have been reported to show an active escape response when approaching a predator tentacle (Trager et al. 1994; Heidelberg et al. 1997). From this point of view, one would expect that IBBS are captured more easily by the corals than live nauplii. But, apparently, IBBS does not trigger the feeding response of the corals to the same extent as live nauplii do. Corals kill their prey by nematocyst discharge, a process that is controlled by both chemical and mechanical factors (Thorington and Hessinger 1988). With respect to the latter, Heidelberg et al (1997) suggested that prey items approaching tentacles with a higher velocity would be captured more easily. When feeding corals with IBBS, it may therefore be beneficial to increase the water flow in the aquarium. This may also reduce the loss of IBBS due to precipitation and stickiness.

The possibility that IBBS lack a chemical trigger can also not be ruled out. Live zooplankters excrete small amounts of amino acids, which have been reported to trigger the feeding response in coral polyps (Mariscal et al. 1968). Lehman and Porter (1973) showed that addition of proline, glutamic acid, aspartic acid and arginine stimulates corals to ingest pieces of filter paper. In this light, there are possibilities to improve Instant Baby Brine Shrimp.

Enriched and medicated nauplii

Although captured with a lower efficiency than non-enriched nauplii, the SELCO-enriched and medicated nauplii were readily consumed by the corals, showing that *Artemia* nauplii can effectively be used as a vector to supply the corals with specific components such as antibiotics or probiotics.

This result provides a useful first step towards development of validated procedures to treat diseased corals with medicated *Artemia*, in analogy with Chair et al (1996), who conducted successful experiments with these antibiotics in fish and shrimp. However, successful application of this technique to corals will depend on several factors that remain to be studied, such as the digestion rate of the antibiotics in the gut of the polyps and the localization of the antibiotics' target. Oral administration of antibiotics may not be the most optimal way to treat diseases that primarily affect the outer cell layer of the coral tissue. In addition, diseased corals may cease their feeding activity.

Nevertheless, the possibilities for coral-specific enrichments are not limited to the addition of antibiotics. Corals are known to be dependent on heterotrophic feeding in order to obtain specific components needed for growth. For example, Allemand et al (1998) suggested that in *Stylophora pistillata*, heterotrophic feeding was the main source for aspartic acid, which is a main constituent

of the skeletal organic matrix of this species. Specific enrichment of *Artemia* nauplii with aspartic acid may reduce the amount of food needed to breed these corals.

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Differential Expression of Soluble and Membrane-bound Proteins in Soft Corals (Cnidaria: Octocorallia)

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Abstract. Current understanding of the ecological role of corals is largely based on extrapolations of physiological processes studied on coral colony fragments as model experimental units. Due to their modular nature, these organisms are assumed to be structurally and functionally homogeneous. Here we address this assumption in an investigation of the distribution of proteins in soft coral tissue regions. This is the first study comparing variations of protein content and protein profiles within colonial cnidarians. *Sarcophyton* sp and *Capnella gaboensis* specimens were sectioned into colony regions with different physiological potential, based on their proximity to endosymbiotic algae and/or direct contact with the external medium. The tissues were homogenized in either 'NP40 buffer' a solution efficient at extracting membrane-bound proteins, or 'Tris buffer' which is efficient at extracting soluble, cytosolic proteins. SDS-PAGE analysis of proteins showed significant intracolonial differences of protein content ($p<0.05$) and differences of protein profile ($p<0.05$). This investigation indicated that the specific proteins have a different pattern of distribution ($p<0.05$) when the tissues are processed using NP40 or Tris buffers. Different spatial expression of proteins suggests regional functional specialization within the structurally repetitive units of these modular organisms.

Keywords. Octocorals, protein, electrophoresis, modular organisms.

Introduction

Whole protein content is routinely measured as an index of physiologically active biomass of cnidarians in numerous investigations such as skeletal and cellular growth (Goreau 1959; Velimirov and King 1979; Barnes 1985; Allemand and Grillo 1992; Tentori et al. 2004); coral-zooxanthellae interactions (Michalek-Wagner and Willis 2001); and expression of specific proteins (Snyder and Rossi 2004; Phelan et al. 2006). Edmunds and Gates (2002) considered soluble protein an acceptable normalizing trait that reflects the spatio-temporal variability of biomass. Tentori and Allemand (2006) questioned the implicit assumptions that protein is equally distributed through coral tissues and interacts uniformly with all the tissues of the experimental coral specimens. Indeed, seasonal and stress-related variability in the protein content of colonial cnidarians has been shown in recent studies (Barneah et al. 2005; Ravindran and Ragukhumar 2006). Further research on the chemical nature of colonial cnidarian tissues is needed to appreciate their functional complexity.

To understand the way the tissues of coral colonies interact with the environment, we need to consider their physical organization. Hard and soft corals adopt a range of growth forms from fine-arborescent to thick-massive structures. Hard and soft coral specimens of comparable dimensions are common occurrence in healthy Indo Pacific reefs, but their internal compositions are remarkably different. All cells in the stony coral body are located close to the external medium and to their symbiotic algae; the tissues spread generally less than 1 mm thick over the skeleton (Quan-Young and Espinoza-Avalos 2006). The widely used water pik method developed by Johannes and Wiebe (1970) amongst coral researchers in the analysis of coral tissues highlights the assumed even distribution of functional components. By contrast, in the soft coral body the polyps form a mass several centimetres thick (Chevalier et al. 1984); the distribution of zooxanthellae is limited by penetration of light into the external tissues (Chin 1996). Clearly, the soft coral morphology increases the resolution needed to study the functional variability of tissues in the modular nature of colonial cnidarians.

The aim of this study is to investigate the spatial distribution of proteins in soft corals. Two zooxanthellate soft corals were studied: *Sarcophyton* sp and *Capnella gaboensis*. Colony regions with different potential of interaction with their endosymbiotic algae and the external medium were analyzed, we refer to these as *functional regions*. We hypothesized that the identified functional regions

would be equipped with different proteinic component. Specific protein bands obtained from tissue homogenates processed under different methods were compared.

Materials and Methods

The experimental organisms

Sarcophyton sp. was collected at 1-3 m depth, 5km off Broome, Western Australia and maintained for two weeks in aquarium conditions: 35 ppt salinity, light intensity of 100 µmol photons m^{-2} s^{-1}, 26°C temp, fed once a week, 30% water exchanged weekly. *Capnella gaboensis* was collected at Fairlight, on the Sydney coast, at 6 m depth, this coral was processed on the day of collection.

Preparation of samples

Tissue samples from various functional regions of one *Sarcophyton* sp. colony and one *C. gaboensis* colony were sectioned as shown in Fig. 1A-B. The tissue homogenates were treated with two buffer solutions: NP40 buffer (40 mM Tris, pH 7.4, 1% Nonidet NP-40, 0.25% cholic acid, 150 mM NaCl, 1mM PMSF, 1 mg ml^{-1} aprotinin, 1 mM NaF) that solubilises membrane-bound proteins; and Tris buffer (40 mM Tris, pH 7.4, 10 mM EDTA, 1 mM PMSF, 1 mg ml^{-1} aprotinin), that solubilises cytosolic water-soluble proteins. Coral samples ranging from 0.2 to 0.9 g wet weight were homogenized on ice, in 0.5 ml of one of the buffer solutions using a Teflon grinder and kept for 30 min in ice and spun at 10,000 rpm, 4°C for 1 min. The coral extracts were stored at –20°C until time of analysis.

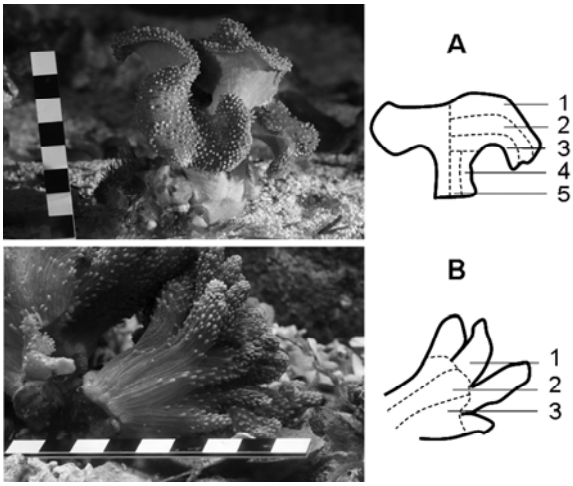

Figure 1. Soft coral colonies and functional regions investigated. (A) *Sarcophyton* sp.: capitulum upper (1); capitulum middle (2); capitulum lower (3); stem surface (4); stem subsurface (5). (B) *C. gaboensis*: lobe (1); stem surface (2), stem subsurface (3). Ruler marks = 1 cm.

Analysis of proteins

Total protein content was measured by colorimetry with bicinchoninic acid (BCA) as standard, and read at 526 nm using a microplate reader. Proteins were analysed by sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE). Molecular marker and 3 replicate samples were loaded onto 12% Tris Glycine gels using 4 % stacking gel and run at 200V for approximately 1 h. The analysis of gel images was performed using the public domain NIH Image program (http://rsb.info.nih.gov/nih-image/). Using as a reference a molecular ladder, selected bands were analyzed and compared across all the resulting gels.

Experimental set up

Total protein content was analyzed in duplicate samples. The electrophoretic analysis was performed in triplicate samples. Two protein bands (approximate MW of 54 and 35 kDa) were compared amongst all treatments in each coral species. The effects of buffer and functional region on these two main specific protein bands were tested by one-way ANOVA.

Results

Intracolonial distribution of proteins

The analysis of tissues showed decreasing protein concentrations from distal to proximal region of the colony and from surface to subsurface of the stem (Fig. 2A-B). The difference of protein content between regions was highly significant in both coral species (*Sarcophyton* sp ANOVA $p<0.001$; *C. gaboensis* ANOVA $p<0.001$). Tissue extracts prepared with NP40 and with Tris buffers showed similar general trends of variation of whole protein content. The two buffers produced a statistically significant difference of protein yield in *C. gaboensis* (ANOVA $p<0.01$) but not in *Sarcophyton* sp (ANOVA $p>0.05$).

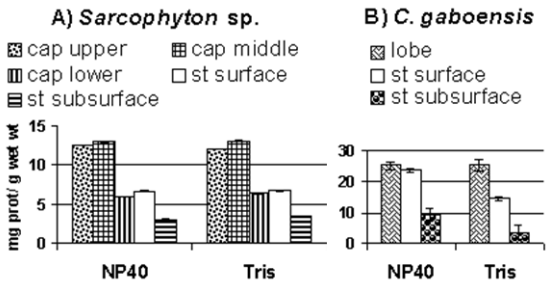

Figure 2. Total protein content in regions of the coral colony processed with NP40 and Tris buffers (n=2). (A) *Sarcophyton* sp. (B) *C. gaboensis*. Abbreviations: cap = capitulum; st = stem (mean ± 1 sd).

Extraction of specific protein bands

SDS-PAGE on coral tissue extracts prepared with NP40 and Tris buffers produced at least 12 protein bands in the molecular weight range 216 to 7 kDa as revealed by Coomassie Blue (Fig. 3A-B). The protein bands with approximate molecular weights of 54 and 35 kDa and three light bands closely set in the range 17 to 7 kDa showed the greatest contrast between the protein profiles obtained by the two methods of extraction. The 54 and 35 kDa bands were analysed by densitometry

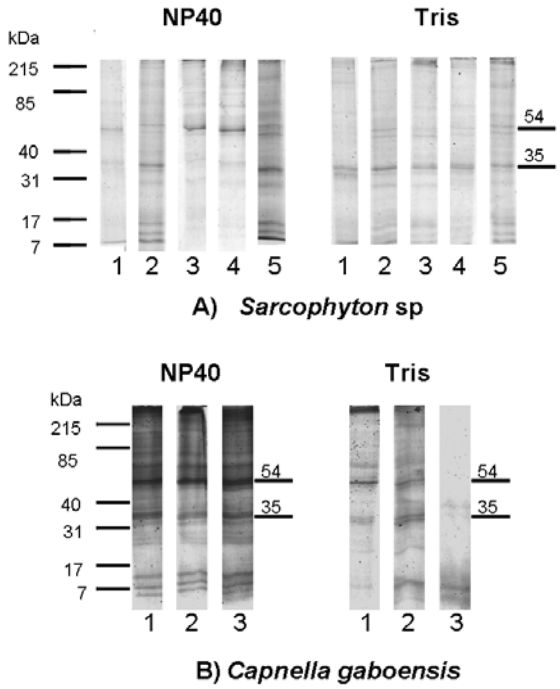

Figure 3. SDS-PAGE protein profiles of soft coral extracts. (A) *Sarcophyton* sp. Regions indicated by tracks: 1 = capitulum upper; 2 = capitulum middle; 3 = capitulum lower; 4 = stem surface; 5 = stem subsurface. (B) *C. gaboensis*. Regions indicated by tracks: 1 = lobe; 2 = stem surface; 3 = stem subsurface.

The density of specific protein bands 54 and 35 kDa was highly variable throughout both coral species. The method of protein extraction also produced significant variation in the content of these protein bands (Figs. 4-5). There were 3 functional regions in which one of the specific protein bands was not detected by one of the methods (54 kDa *Sarcophyton* sp. capitulum upper region in Tris; 35 kDa *Sarcophyton* sp. capitulum lower region in NP40; 54 kDa *C. gaboensis* stem subsurface region in Tris). The interaction between regions and extracts was significant in all cases except for protein band 35 kDa in *C. gaboensis* (see Tables 1 and 2).

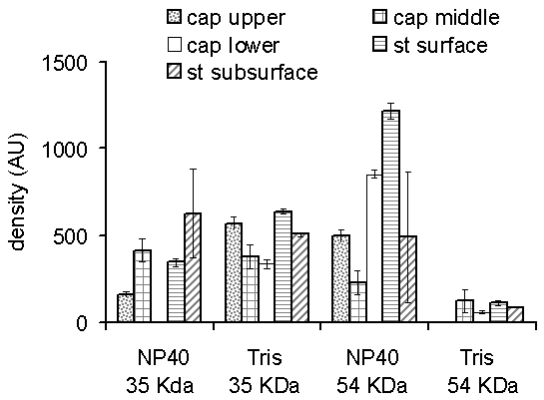

Figure 4. *Sarcophyton* sp. Comparison of two protein bands obtained by SDS-PAGE in coral samples prepared with NP40 and with Tris buffers (n=3). (A) Protein band 54 kDa; (B) Protein band 35 kDa. Density of bands is reported in arbitrary units (mean ± 1 se).

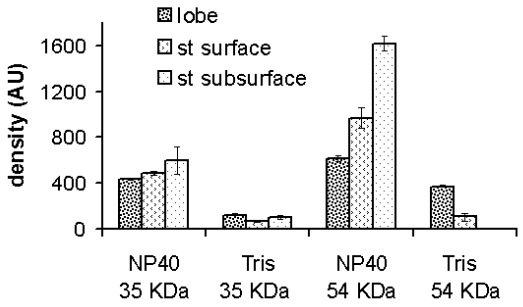

Figure 5. *C. gaboensis*. Comparison of two protein bands obtained by SDS-PAGE in coral samples prepared with NP40 and Tris buffers (n=3). (A) Protein band 54 kDa; (B) Protein band 35 kDa. Density of bands is reported in arbitrary units (mean ± 1 se).

Discussion

This investigation contains the first comparative data of whole protein content as well as specific protein bands in tissues from various regions of soft coral colonies. The application and comparison of two different buffer solutions in the extraction of cnidarian proteins is also tested for first time.

In any organism, protein half-lives range from minutes to months depending on their function and location; their continuous renewal is the basis for biological adaptation (Hawkins 1991) yet few studies have considered the spatial variation of proteins in colonial cnidarians. The variation of protein content between polyp and stem regions in the soft corals *Sarcophyton ehrenbergi* measured as elemental carbon-to-nitrogen ratios (Tentori et al. 1997) and *Litophyton arboreum* measured as sclerite-to-protein weight ratios (Tentori et al. 2004) indicate that distal tissues are richer in protein than are proximal tissues. Protein content (measured as protein-to-biomass weight ratios) of soft corals reported in the present study confirm those results. Furthermore, our data

show significant differences in protein content throughout the colony. These data are indicative of different functional potential amongst the tissues involved.

Variability of Specific Proteins

Research on protein diversity of colonial cnidarians highlights the dynamic metabolism of these organisms. Electrophoretic protein profiles have been generated for various cnidarian species identifying stress proteins associated with heat shock (Black et al. 1995; Sharp et al. 1995); space competition (Rossi and Snyder 2001); dietary changes (Rossi et al. 2006); zooxanthella acquisition (Barneah et al 2005); and microbial infection (Ravindran and Raghukumar 2006). In these studies two important aspects of the temporal variability of specific proteins in colonial cnidarians are appreciated: 1) the variation of specific proteins does not match the trend of variation of whole protein content (Black et al 1995; Ravindran and Raghukumar 2006; Rossi and Snyder 2001; Rossi et al 2006); 2) a change of specific proteins can be induced within hours in the laboratory or within several weeks under natural environmental conditions (Black et al 1995; Sharp et al. 1995; Rossi and Snyder 2001; Barneah et al. 2005).

Table 1. *Sarcophyton* sp. one –way ANOVA. Specific protein bands in five functional regions (capitulum upper, capitulum middle, capitulum lower, stem surface, stem subsurface) in two different homogenate extracts (NP40 and Tris).

Source of variation	df	54 kDa F	54 kDa *P*	35 kDa F	35 kDa *P*
Extract	1	59.2	<0.001	10.9	<0.01
Regions	4	5.3	<0.01	6.2	<0.05
Interaction	4	5.1	<0.01	3.9	<0.05
Within	20				
total	29				

Table 2. *C. gaboensis* one-way ANOVA. Specific protein bands in three functional regions (lobe, stem surface, stem subsurface) in two different homogenate extracts (NP40 and Tris).

Source of variation	df	54 kDa F	54 kDa *P*	35 kDa F	35 kDa *P*
Extract	1	515.4	<0.01	102.0	<0.01
Regions	2	24.2	<0.01	1.4	>0.05
Interaction	2	99.2	<0.01	1.9	>0.05
Within	12				
total	17				

The spatial variability of proteins in cnidarians is less well documented. Rossi and Snyder (2001) reported the variation of stress proteins amongst tentacles of single polyps in anemones and corallimorpharians, they pointed out the differences between whole-organism and selected-tissue responses. Given the apparent morphological simplicity of colonial cnidarians, their protein analysis is commonly performed on whole experimental specimens or unspecified fragments of those specimens. We suggest that detailed knowledge of the spatial variability of proteins within the cnidarian colony tissues could increase the resolution of the experimental response sought in physiological investigations.

In the present study, the protein bands, of approximate molecular weights of 54 and 35 kDa, stand out as a common feature in both *Sarcophyton* sp and *C. gaboensis* suggesting their involvement in a cellular activity or structural requirement common to both species. Yet the variable abundance of these protein bands indicates contrasting needs amongst the various functional regions analysed. Our results are evidence of tissue specialization in organisms that have been largely regarded as morphologically and functionally uniform.

Soluble and membrane-bound protein distribution

Specific protein analysis is commonly performed on the soluble fraction of tissue homogenates processed either in Tris-HCl (Black et al 1995; Sharp et al 1995; Bythell et al 1995; Barneah et al 2006) or in phosphate buffers (Harithsa et al 2005; Ravindran and Raghukumar 2006; Takeda et al 2000; Rossi and Snyder 2001; Rossi et al 2006). Hashimoto et al. (2005) analysed both soluble and insoluble proteins obtained from Tris extracts of anthocodia (distal region of the polyp) of the soft coral *Clavularia viridis*; they demonstrated a different protein profile for each of the two fractions. Our results agree with that general finding.

The use of NP40 allows detection of membrane-bound proteins. To our knowledge, this is the first application of NP-40 to obtain proteins from cnidarian tissues. Our study revealed a significant difference of the protein profile of *Sarcophyton* sp and *C. gaboensis* depending on the extraction buffer used and colony region analysed (Tables 1 and 2).

The existence of different tissue regions in soft coral colonies is acknowledged in early taxonomic descriptions highlighting the site-specific shape of their sclerites (Verseveldt 1977). Furthermore, there is evidence of functional gradients in the soft coral body associated with feeding (Schlichter 1982; Schlichter et al. 1983), secondary metabolite production (Maida et al. 1993; Van Alstyne et al. 1994), gastrovascular circulation (Gateño et al. 1998), cell growth and tissue repair (Tentori et al. 2004), characteristics that are no doubt linked to the protein profile of the tissues involved. We suggest soft corals as model organisms that offer a natural experimental setting to enhance spatial resolution in cnidarian physiology.

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Practical applications of contaminant-free *Symbiodinium* cultures grown on solid media

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Abstract. Symbiotic dinoflagellates in the genus *Symbiodinium* are critical to the success of scleractinian reef corals in shallow tropical seas. These symbionts are commonly isolated from hosts and cultured separately in liquid media (f/2 or ASP8a), but initial isolations can be prone to abundant contaminants that can persist long-term in culture. To help remove these contaminants, we developed a solid growth substrate composed of 1% agar in f/2 medium, supplemented with a variety of antibiotics, to help isolate individual clones and establish new "axenic" cultures. We found that an antibiotic cocktail of kanamycin (50 μgmL^{-1}), ampicillin (100 μL^{-1}) and streptomycin (50 μgmL^{-1}) was the most effective at eliminating visual signs of contamination without apparent harm to a variety of *Symbiodinium* in culture. Photophysiological measurements of *Symbiodinium* grown on agar plates, taken using an Imaging Pulse Amplitude Modulated (I-PAM) fluorometer, were comparable with those grown in liquid f/2 medium, both with and without antibiotics. *Symbiodinium* cultures grown on solid substrates supplemented with antibiotics are useful for: (1) isolating individual cells or clones for subsequent applications; (2) establishing and maintaining "axenic" cultures, free of observable contaminants; and (3) directly comparing (on the same plates) the photophysiology of different cultures using an I-PAM fluorometer.

Key words: *Symbiodinium*, coral, physiology, culture, I-PAM fluorometry

Introduction

Scleractinian reef corals depend on symbiotic dinoflagellates in the genus *Symbiodinium* for their metabolic needs (Muscatine 1967). Currently, 8 clades (A-H) are recognized, each of which contains multiple sub-types (Coffroth and Santos 2005; Pochon et al. 2006). This genetic diversity could provide a mechanism for corals to adapt or acclimatize to environmental changes, provided that genetic diversity in the symbionts translates to different physiological capabilities for the coral host. Studies of symbiont photophysiology may therefore help provide insight as to whether symbiont diversity can help corals survive environmental change.

Cultures of *Symbiodinium* isolated from different invertebrate and protist hosts are usually established and maintained in liquid media (f/2 or ASP8a), but are often prone to abundant contaminants that can be difficult to remove. Antibiotics and repeated dilutions have been used to eliminate some contamination; however, this process is time consuming and inconsistent (Anderson and Kawachi 2005). In addition, isolating a single cell of interest is difficult using traditional methods, which involve using capillary action to isolate a cell in an ultrafine glass pipette (Guillard 2005). Agar blocks set onto liquid media have previously been used to select for single transgenic *Symbiodinium* cells, but this method required daily additions of fresh liquid media and antibiotics (ten Lohuis and Miller 1998).

Agar plates have been routinely used for culturing microalgae, but only rarely for dinoflagellates (Schoenberg and Trench 1980; Guillard 2005). Growing *Symbiodinium* spp. on agar plates resolves some of the issues mentioned above, and agar plates with host homogenate have been successfully used to isolate new types of *Symbiodinium* in clades C and D from the giant clam, *Tridacna crocea* (Isikura et al. 2004).

Here we show how growing *Symbiodinium* on a solid medium of 1% agar, supplemented with f/2 medium and a combination of antibiotics, helps isolate single cells, eliminate microbial contaminants, and facilitate photophysiological measurements using an Imaging Pulse Amplitude Modulated Fluorometer (I-PAM: Walz, Inc., Effeltrich, Germany).

The I-PAM is a convenient tool for measuring and visualizing photosynthetic parameters such as effective quantum yield (α), electron transport rate (ETR), and quantum yield of Photosystem II (as the ratio of variable fluorescence to maximum fluorescence, or F_v/F_m). Together, these parameters

have proven useful in assessing photosynthetic response to light limitation, the ability of symbionts to tolerate changes in light over a short period of time, and overall photosynthetic efficiency (Rolfe and Sholes 1995; Ralph and Gademann 2005).

Material and Methods

Maintenance of algal cultures

Algal cultures, supplied by S. Santos (Auburn University, USA), were maintained in liquid f/2 media or solid media (described below). Incubator settings were 25.5°C, 12h L/D cycle. Liquid media were changed monthly. Cultures were re-plated monthly, while still keeping the original plates.

Production of agar plates

A solid growth medium was made by autoclaving 1% bacto-agar (Difco) in 20 mL filtered seawater. Once the mixture had cooled to <55°C, 0.4 mL of 50X f/2 medium (Sigma) was added, and the mixture was then poured onto plates (100mm diameter) and allowed to cool. After 24h, 10-30 µL aliquots of 19 different *Symbiodinium* cultures were spread onto the plates (Table 2). All plates were sealed with parafilm.

Testing resistance to antibiotics

After one month growing on the solid medium, we tested the effectiveness of antibiotics in removing contaminants in 10 cultures with the highest levels of microbial contamination. With a bacterial loop, we removed approximately 10 µL of *Symbiodinium* from each plate (together with their associated contaminants), mixed this with 200uL filtered seawater, and spread this inoculate onto fresh plates to which we added a variety of antibiotics (Table 1). These plates were prepared as outlined above, with the antibiotics added with the f/2 medium as the agar cooled. We also inoculated control f/2 plates with no antibiotics. The relative growth of microbes and *Symbiodinium* was recorded after 3, 7 and 14 days, and periodically for 5 months thereafter.

Elimination of contaminants

Symbiodinium colonies growing on the antibiotic treatment showing the least contaminants (a cocktail of kanamycin, ampicillin and streptomycin, "KAS", see Table 1) after 2 weeks were picked with a bacterial loop and re-streaked onto fresh KAS-treated plates. After 1-3 weeks, if contamination persisted, single *Symbiodinium* cells were picked from plates, using a straight wire under a microscope, and then re-streaked onto new plates.

Photophysiological comparisons of symbionts

We compared the photophysiological parameters of *Symbiodinium* culture Zs (in clade A) grown in liquid f/2 (n=5) with the same culture grown on f/2 plates (n=2) and f/2 plates with KAS (n=5). Cultures were grown from contaminant-free clones picked from a KAS plate.

Table 1: Growth of *Symbiodinium* and bacterial contaminants after 3-14 days under different antibiotic applications: + medium growth, ++ high growth, – low growth, 0 no visible growth.

Growth of *Symbiodinium* and bacterial contaminants under different antibiotic

Antibiotic	Concentration (µg/mL)	Bacteria	*Symbiodinium*
Erythromycin	50	+	-
Amph B	300	+	+
	30	+	+
Chloramphenicol (C)	50	0	0
	25	0	0
	5	-	-
Doxycycline	50	+	-
Tetracycline	50	+	-
Streptomycin (S)	50	+	+
Ampicillin (A)	3,000	+	+
Pen Strep	300	+	+
	100	+	+
Kanamycin (K)	3000	+	+
	300	+	+
	30	+	+
G418	3,000	+	+
	1,000	+	+
	300	+	+
	100	+	+
K+A	50/100	-	+
K+A+S	50/100/50	-	++
K+A+S+C	50/100/50/5	-	-
None	0	+	+

I-PAM measurements were taken after 19 days. Cultures were dark adapted for 20 min before induction curves were measured, and then returned to the incubator for 20 min before rapid light curves were measured.

The maximum electron transport rate (ETR_{max}) and the effective quantum yield (α) were calculated by fitting rapid light curves to a double exponential decay curve in Sigma Plot (Ralph and Gademann 2005). Photosynthetic efficiency (F_v/F_m) was obtained from induction curves. For solid plates, we compared photosynthetic parameters extracted from the whole plate with those obtained from the average of 5 random points on the plate. For the liquid medium we measured parameters from the whole culture (Fig. 1). Data were compared using a one-way ANOVA followed by post-hoc Tukey-Kramer HSD tests if significant differences were found.

Results

Resistance to antibiotics

Most liquid stock cultures are prone to high levels of microbial contamination that cannot be controlled with a single antibiotic application. Cultures maintained on f/2 plates with KAS remained free of contaminants for at least 5 months, with no visual signs of bacterial growth. Agar plates with KAS show the highest relative growth for symbionts but the lowest bacterial growth (see Table 1). In addition, bacterial growth was easily reduced by physically removing symbionts from bacteria by picking clones and re- streaking. There was 100% mortality for both symbionts and bacteria on chloramphenicol plates after 3 days. Kanamycin and G418 had a high relative growth for at least one bacterial morphotype (based on colony shape and color), even at 3 $mgmL^{-1}$. Some cultures on KAS plates still showed some bacterial contaminants after 5 months; however, contamination was approximately an order of magnitude less on a per-cell basis than the same culture grown in control f/2 media (solid or liquid) with no antibiotics (see Table 1).

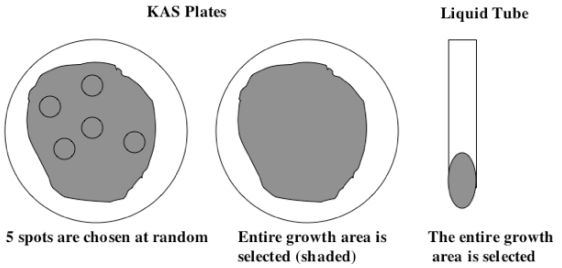

Figure 1: Two methods for IPAM measurements were used: selecting 5 spots at random of growth area, or selecting the entire growth area referred to as "whole plate." The entire liquid area (20 ml) was selected for liquid tube I-PAM measurements. Lids were removed from plates under the IPAM.

Many types of *Symbiodinium* can be grown on agar plates (Table 2), but in this study, members of clades A and C had the highest growth rates. Most of the 19 *Symbiodinium* types we tested on agar plates showed positive growth on this medium. Some were difficult to maintain long-term, particularly those in clade D. However, the majority of cultures were easily maintained on agar plates for >5 months and repeatedly used to create new stocks.

Photophysiological comparisons

The two sampling methods for taking I-PAM measurements on plates (5 random spots vs. whole plate), produced comparable mean values of ETR_{max} and α (P> 0.7292 and P> 0.9998, determined by a one- way ANOVA, respectively), both on f/2 only plates and f/2 + KAS plates. Further, following the ANOVA test, the F_v/F_m, values as shown by a post hoc test(Tukey-Kramer HSD test) displayed no significant difference within the same type of plates for either of the two sampling methods (Fig. 2). In contrast the post hoc test did show a significant difference between plates and liquid tubes for F_v/F_m Variability in all parameters was less using the "random spot" method, compared to "whole plate" method (error bars in Fig. 2). F_v/F_m values were significantly lower for solid media on plates,

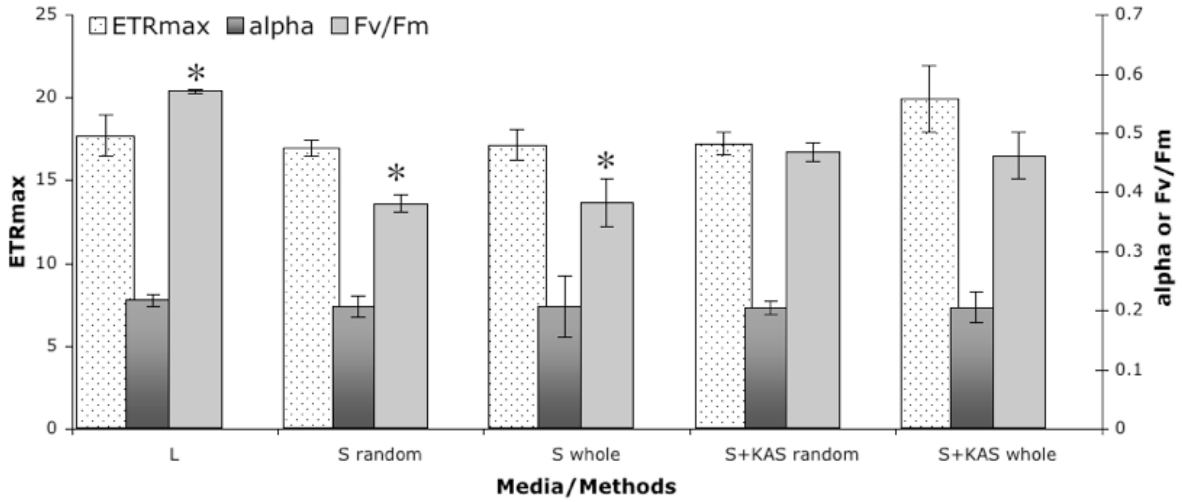

Figure 2: Comparison of photophysiological parameters (ETR_{max}, α and F_v/F_m) for *Symbiodinium* culture Zs (clade A) grown on three media types: L (liquid f/2), S (solid agar f/2) and S+KAS (solid agar f/2 supplemented with KAS) (whole plate and 5 spots). Error bars represent standard error of the appropriate mean value. A Tukey-Kramer HSD test revealed significant differences between treatments for parameters marked with an asterisk (*). Values not marked with an asterisk are not significantly different among methods and media.

compared to liquid media in tubes (P>0.0007; Fig. 2). All other comparisons showed no statistical differences from each other.

Discussion

General plate applications

Our results indicate that growing *Symbiodinium* on sold agar plates supplemented with f/2 and antibiotics is useful for the following applications: isolating single cells and clones, purifying cultures and reducing contamination, and undertaking comparative photophysiological studies. Current techniques to remove microbial contamination include repeated antibiotic doses applied on a weekly, or even daily, basis. This can be expensive and time-consuming. Using f/2 + KAS plates, the use of antibiotics is limited to 2-3 applications over a >5 month period. Transferring symbionts to new media is only necessary when plates begin to dry out (which typically occurs after a few months), and is mainly done as a precautionary step to avoid dessication and and self-shading of cultures. In addition, microbial contamination can be readily detected (by eye or microscope) and removed. In liquid media, contamination is much harder to detect, and can be done only by determining relative "cloudiness." Our cultures remained completely free of any visible contamination (via microscope) for >5 months on the same KAS plates, and remained sufficiently viable to establish new cultures at the end of this period. A potential pitfall of any plate-based method is local depletion of either antibiotics or nutrients over time, leading to mortality or decreased growth rates. More studies are needed to determine the long-term viability of *Symbiodinium* maintained on the same plate. Another potential problem is that sub-culturing from plate to plate may result in genetic drift. This might be reduced by mixing multiple clones picked from plates to establish new cultures.

Table 2: Types of *Symbiodinium* grown on agar plates, with their invertebrate host and geographic location. * denotes contaminate free cultures for over 5 months, + denotes slow growing, - denotes low amounts of contaminants.

Types of Symbiodinium cultures grown on agar plates

Culture ID/Clade	Invert Host	Geo Location	Status
FLAp1/A	*Aiptasia pallida*	Caribbean	*
T/A	*Tridacna gigas*	Indo-Pacific	-
719/A	*Pseudoplexaura porosa*	Caribbean	*
Zs/A	*Zoanthus sociatus*	Caribbean	*
Y109/A	*Unknown host*	W. Pacific	-
Mf 12.5f/B	*Montastraea faveolata*	Caribbean	-
Pd/B	*Pocillopora damicornis*	C. Pacific	-+
703/B	*Plexaura kuna*	Caribbean	-
PurPflex/B	*Plexaura flexuosa*	Caribbean	*
Mf 1.56/B	*Montastrea faveolata*	Caribbean	-
Mf 6.1T/C	*Montastraea faveolata*	Caribbean	-
Mv/C	*Montipora verrucosa*	C. Pacific	*
PtBr/C	*Briareum* sp.	Caribbean	*
Pa 45a/C	*Porites astreoides*	Caribbean	-
Mf10.8a/D	*Montastraea faveolata*	Caribbean	-
A008/D	*Acropora sp-4*	W. Pacific	-+
A013/D	*Porites annae*	W. Pacific	-+
Ap37/D	*Unknown anemone*	W. Pacific	-
CCMP421/E	*Free living*	W. Pacific	-

An additional potential application of agar plates and antibiotics is the selection of genetically transformed *Symbiodinium*. Chloramphenicol plates were very effective in killing *Symbiodinium* (and bacteria), and might therefore be used as means of selecting for successful transformants.

I-PAM specific plate applications

ETR_{max} and α were not significantly different for tubes or plates (with or without antibiotics), indicating that the plate method is equivalent to the traditional liquid tube method for rapid light curve measurements. We recommend that the "whole plate" method be used for large-scale experiments with >5 replicates, since the method is more rapid while still yielding results that are comparable to the "random spot" method. For studies with few replicates, the "random spot" method should be used to reduce error.

We found plates easier to manipulate than liquid media since there was no need to separate clumps and the entire plate could be visualized using the I-PAM. Additionally, individual clones grown on plates can be selected by the I-PAM as "Areas of Interest", allowing within-population comparisons to be made. In liquid media, where symbiont populations are mixed, these distinctions cannot be made.

Solid plates also allow clones with certain characteristics (such as high growth rate, varying F_v/F_m rates etc) to be selected from a population. Finally, different types of *Symbiodinium* can be compared side-by-side on the same plate (we have cultured up to 4 cultures in different quadrants of the same plate) for physiological comparisons among types. This helps reduce error within treatments.

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Differences in the distribution of *Symbiodinium* spp. among morphotypes and genotypes of *Porites panamensis* from the Gulf of California, Mexico

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Abstract. Cnidarian-algal symbioses are important in sustaining coral reef ecosystems. Little is known about how these associations respond ecologically and evolutionarily to environmental change. We examined the diversity of zooxanthellae populations in two morphotypes of *Porites panamensis* in the southern Gulf of California. Additionally, we analyzed the host genetic information by allozyme electrophoresis in order to demonstrate if the species of symbiont corresponds with the genotype and/or morphotype of the host individual. The specimens (N = 20) were collected from shallow coral communities (1-2 m). *Symbiodinium* C66a associated with columnar colonies while C66 occurred commonly in colonies with mounded or massive morphologies. Both colony forms associated with C1. Higher allelic diversity and different symbiont clades were found in columnar colonies, relative to massive colonies in the same habitat. Certain host genotypes associated specifically with a particular *Symbiodinium* type. These preliminary findings indicate that host-symbiont co-evolution is important in shaping partner combinations and that the mode of symbiont acquisition by maternal or vertical transmission facilitates this process.

Key words: host-symbiont specificity, *Symbiodinium*, host-genetic data, species complex, scleractinian coral

Introduction

Partner specificity is important to the biology of all reef corals maintaining obligate associations with endosymbiotic dinoflagellates in the genus *Symbiodinium* (zooxanthellae) (Baker 2003; Stat et al. 2006). Ecological and physiological studies indicate that tolerance to low and/or high light and temperature differs among *Symbiodinium* (Iglesias-Prieto et al. 2004; LaJeunesse et al. 2008). Hosting a particular symbiont may therefore determine the longevity of the colony when exposed to episodes of stress. Of critical interest is determining how changing environmental conditions affect the stability and evolution of these partnerships over time (LaJeunesse 2004; Stat et al. 2006). Initial analyses of corals in the Gulf of California (GC), have showed that they maintain highly specific and stable associations, despite wide seasonal fluctuations in light and temperature (Iglesias-Prieto et al. 2004; LaJeunesse 2004; LaJeunesse et al. 2008).

The coral *Porites panamensis* is widely distributed in the Eastern Pacific. In the Gulf, this species has two colonial morphologies with slight differences in corallite morphology (Ketchum and Reyes-Bonilla 2001), ability to recover from physical damage (Paz-García and Reyes-Bonilla 2006), and vertical distribution (López-Pérez et al. 2003; Paz-García and Reyes-Bonilla 2006), and appear to be genetically distinct (Paz-García et al. 2008b). These data suggest that several factors could explain the variation observed in both morphotypes (Paz-García et al. 2008b). *Porites panamensis* harbors different symbiont types (LaJeunesse et al. 2008), but it is unknown whether the distribution of these symbionts relates to host-morphotype. Our aim was to examine the diversity of *Symbiodinium* in these two morphotypes of the coral *P. panamensis* in the southern Gulf of California. Additionally, We analyzed the host genotypes by allozyme electrophoresis in order to demonstrate if the species of symbiont corresponds with the genotype and/or morphotype of the host individual. This study is among the first to provide evidence that host genotype dictates the distribution of different symbiont types.

Material and Methods

Field Work. The collections were conducted in May 2006 in two locations in the southern Gulf of

California, Mexico (Fig. 1). The columnar morphotype of *P. panamensis* is more prevalent at Punta Gaviotas, the massive one at Punta Galeras (Paz-García and Reyes-Bonilla 2006). The specimens (n = 10 for each morphotype) were colleted from shallow coral communities (1-2 m).

Figure 1: Map showing the locations, in the south of the Gulf of California.

Symbiont Identification. Coral fragments were stripped of tissue using an airbrush and the symbiont cells isolated as described by LaJeunesse et al. (2003). Algal pellets were transferred to 1.5 mL microcentrifuge tubes and preserved in 20% dimethylsulfoxide (DMSO), 0.25 mol L^{-1} ethylenediamenetetraacetic acid (EDTA), in sodium chloride-saturated water solution (Seutin et al. 1991). The genetic identity of the *Symbiodinium* populations in each sample was later analyzed via PCR-denaturing gradient gel electrophoresis (DGGE) of the ITS regions 1 and 2 as described by LaJeunesse et al. (2008).

Host genetic analysis. We conducted a coral tissue extraction as described by Paz-García et al. (2008a). We determined the concentration of total proteins from each sample by Bradford's method (Bradford 1978) and 25 μg were used for the analysis of each enzyme system. Allozyme analysis was carried out using polyacrylamide gels. Four enzyme systems were used: LGG (E.C.3.4.11.1), ME (E.C.1.1.1.40), GDH (E.C.1.4.1.3) and EST (E.C. 3.1.1.1).

To examine the differences between host morphotype and symbiont ITS type, we scored and compared the frequencies of allozyme alleles between colonies that were either columnar or massive and/or associated with C66, C66a, or C1. Host allelic and genotype frequencies were tested by the Markov chain method (Raymond and Rousset 1995). Host unbiased genetic distance (Nei 1978) was used to carry out cluster analysis among clade groups.

Results

Six colonies of columnar *P. panamensis* associated with *Symbiodinium* C66a while the other four colonies harbored C1. The distribution of these combinations appeared random. All 10 shallow massive colony morphotypes collected from Punta Galeras associated with C66 (Fig. 2). A single symbiont type was detected in each colony analyzed.

During the animal genotyping, two to four alleles were found for each allozyme locus analyzed. Higher allelic diversity was found in columnar colonies relative to massive colonies (Fig. 3). One genotype of locus LGG-1 and three genotypes of locus ME-1 were observed in colonies associating with a particular symbiont type (Table 1). Significant differences in host allelic and genotype frequencies were determined by Markov chain analyses of columnar C1 and massive C66 colonies (Table 2), but allelic and genotype diversity in colonies with C66 and C66a were not statistically different or were columnar colonies with C66a or C1 (Table 2). The relationship between host-genotype and symbiont type was supported by cluster analysis based on host Nei's unbiased genetic distance that showed massive colonies with C66 and columnar colonies with C66a clustered together before joining columnar colonies hosting C1 (Fig. 4).

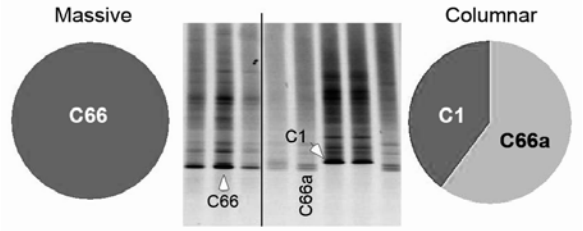

Figure 2: ITS-DGGE fingerprinting analysis of *Symbiodinium* spp. identity and type frequencies in each host morphotype.

Table 1. Host genotypes compared with the *Symbiodinium* type harbored by *P. panamensis* individuals. Animal genotypes observed with only one symbiont type are in bold.

	Massive C66	Columnar C66a	Columnar C1
ME-1	AA, AB	AA, AB	**BB**
GDH-1	AA, BB	AA, BB	BB
GDH-2	AA, BB	AA, BB, CC	AA, BB, CC
EST-1	BB, BC, CC	BB, BC, CC	BC, CC
LGG-1	CC, **DD**	**AA**, **AB**, CC, **CD**, DD	**BB**, CC

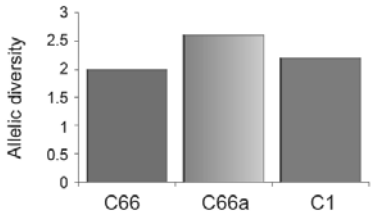

Figure 3: Host allelic diversity based on the symbiont type hosted. Massive morphotype (C66), Columnar morphotype (C66a and C1).

Table 2. Allelic (below diagonal) and genotypic (above diagonal) host differentiation frequency test.

	Massive C66	Columnar C66a	Columnar C1
Massive C66	——	*NS*	*
Columnar C66a	*NS*	——	*NS*
Columnar C1	**	*NS*	——

*X^2=21.378 10 df, p=0.018. **X^2=38.810 10 df, p<0.001.

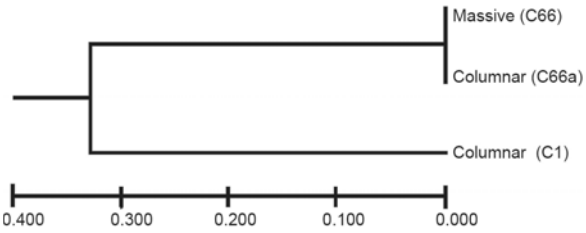

Figure 4: Cluster analysis based on host Nei's unbiased genetic distance.

Discussion

Inter-colony variability in symbiont type among colonies of *Porites panamensis* appears to be influenced by environmental and biological factors. Five different *Symbiodinium* types are known to associate with *P. panamensis* in the Gulf of California (LaJeunesse et al. 2008). Depth strongly influences the frequency of occurrence of particular symbionts in populations of host individuals. *Symbiodinium* C1 is prevalent among colonies living below 2 meters. At depths below 5 meters colonies are occasionally found associating with C75. Closely related C66, C66a, and C66b occur in *P. panamensis* living in shallow environments (0.5 and 1.5 m). These ecological patterns suggest that significant differences in physiology exist among Clade C *Symbiodinium* (Sampayo et al. 2008).

Columnar and massive morphotypes of *P. panamensis* also exhibit differences in vertical distribution throughout the Gulf (López-Pérez et al. 2003; Paz-García and Reyes-Bonilla 2006). Columnar colonies are more limited in their distributions to shallow habitats, typically 0.5 to 4.0 meters from the surface. While these data are limited, the presence of a particular symbiont does not explain this pattern of zonation.

Specific host-symbiont combinations involving *Symbiodinium* adapted to different light regimes may result in ecological zonation along an irradiance gradient (Iglesias-Prieto and Trench 1994; Iglesias-Prieto et al. 2004). This coral belongs to one of the few genera that produce eggs containing symbiont cells (Richmond and Hunter 1990; Glynn et al. 1994). Therefore, the presence of a high or low light-adapted symbiont may directly affect the differential success of larvae settling in deep or shallow environments. Further understanding of the physiological performances of these different host-symbiont combinations (holobionts) under different environmental conditions would help explain these ecological patterns.

There appears to be a close correspondence with symbiont identity and host genotype (Fig. 4). While the columnar or massive morphology does not appear to influence symbiont distribution, the presence of C66 or C66a in hosts with similar genotypes suggests that host genetic background is important to specificity. While external physical factors affect the symbioses in some hosts (Rowan et al. 1997), host-symbiont specificity plays a crucial role in dictating the distribution of certain partner combinations.

Thoroughly describing the ecological and geographic distributions of specific host-symbiont combinations is important for ultimately understanding co-evolutionary processes that occur between host and symbiont lineages. Associations with C1, C66 (a and b variants), and C75 are unique for Indo-Pacific *Porites* (LaJeunesse 2004). The evolution of unusual host-symbiont combinations is probably influenced by populations living for many generations in isolation while subjected to wide ranges in temperature and water clarity, as is characteristic of the northern Eastern Pacific. The presence of different symbionts among colonies of *P. panamensis* may provide the natural variation that may eventually be important in determining how these coral populations respond to selection pressures created by global warming.

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Reference gene selection for qRT-PCR analysis of the Hawaiian coral *Pocillopora meandrina* subjected to elevated levels of temperature and nutrient

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Abstract Quantitative reverse transcriptase PCR has several variables that need to be controlled for to obtain accurate estimates of gene expression data. The use of a single reference gene is a common method to normalize mRNA fractions and obtain reliable quantitative expression measures. However no universally appropriate reference gene exists that is constitutively expressed in all tissue types and all experimental conditions. One plausible solution for this problem is to use a set of reference genes for normalization that display minimal variation across all treatments. Past gene expression studies have often employed the use of a single reference gene. As the number of gene expression studies in reef-building coral is expected to increase, a set of suitable reference genes is needed for normalization. This study characterized the expression of five candidate reference genes for the scleractinian coral, *Pocillopora meandrina*, after chronic elevated nitrogen followed by increased seawater temperature. Two separate gene ranking assessment software programs were used to rank candidate genes by expression stability. The Brn1, β-actin, and 18S rRNA genes were the most stably expressed genes in *P. meandrina* subjected to long-term elevated nitrogen followed by an acute increase in temperature.

Key words: quantitative RT-PCR, gene expression, coral

Introduction

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) is commonly used in biological applications for accurate quantification of gene expression changes between tissues, disease states or treatments (Bustin and Nolan 2004). Several variables between tissues or cells need to be controlled in qRT-PCR including the amount of starting material, differences between tissues or cells, quality of the RNA, and reverse transcription efficiency (Vandesompele et al. 2002). Various strategies have been used to control for the previously stated variations within qRT-PCR including techniques such as standardization to total RNA or to an endogenous reference gene.

Standardization to total cellular RNA can pose problems because it is difficult to quantify small amounts of RNA, and the variance present in reverse transcription and PCR efficiency are not considered (Huggett et al. 2005; Wong and Medrano 2005). In addition, the process of normalizing to total RNA assumes that rRNA is directly proportional to mRNA and does not vary between individuals, tissues, or cell cultures, or under different experimental conditions (Bustin and Nolan 2004). Alternatively, variations can be controlled for by normalization of the target gene to an endogenous reference gene. This comparison allows for a normalized expression value of the target gene that is independent of the quality and quantity of starting RNA and the efficiencies of the reverse transcriptase reactions (Huggett et al. 2005).

The most important criterion of appropriate reference genes in qRT-PCR is that these genes are expressed constitutively in cells irrespective of experimental conditions. In the past it was recommended that if it was not possible to find a constitutively expressed reference gene, it is an option to apply one of several frequently used genes as a control to normalize expressions of other genes against (Bustin 2000). In spite of this, there has been evidence that reference genes can be regulated under different conditions (Bustin 2000; Vandesompele et al. 2002). This means that although use of a referenced "stable" gene can be consistent for one experiment and one type of tissue, there is no universally perfect control gene that is perfect for all tissue types or treatments (Brunner et al. 2004; Vandesompele et al. 2002; Andersen et al. 2004).

qRT-PCR has been used in only a handful of anthozoan and hydrozoan gene expression studies (Yanze et al. 1999; Mitchelmore et al. 2002; Loram et al. 2007; Rodriguez-Lanetty et al. 2007). Many of these studies used a single reference gene to normalize the expression of their target genes. It was

recently reported that experiments that used a single reference gene to normalize expression of target genes let to erroneous quantifications (Vandesompele et al. 2002). Erroneous quantifications occur because gene expression of the control can result in unrecognized or unexpected changes (Vandesompele et al. 2002; Szabo et al. 2004). One probable solution for this problem is to use a set of reference genes for normalization that display minimal variation across the treatments included in the experiment (Vandesompele et al. 2002). There are several existing statistical analysis software programs to analyze the stability of reference genes (Pfaffl et al. 2001; Vandesompele et al. 2002; Andersen et al. 2004) and since their arrival they have been used with increasing regularity (Etschmann et al. 2006; Jorgenson et al. 2006; Nailis et al. 2006; Willems et al. 2006; Maccoux et al. 2007; Tang et al. 2007; Peltier and Latham 2008). In this study two popular statistical analysis software programs were used to identify the most stable candidate reference genes. The first program, GeNorm, calculates the most stable genes from a set of candidate reference genes based on the average pair-wise variation of a single gene compared to all other reference genes in the set (Vandesompele et al. 2002). The second program, NormFinder, evaluates the gene expression variability of reference genes within treatments compared to variation among treatments for each gene (Andersen et al. 2004). Both programs allow the ordering of candidate reference genes based on relative expression stabilities, referred to in this paper as "rank," through the use of a mathematical evaluation of expression data.

A recent study by Rodriguez-Lanetty et al. (2007) used GeNorm analysis to identify β-actin, ribosomal protein L12 and Poly(a) binding protein as three control genes determined to be stable from a cDNA microarray platform constructed from the sea anemone, *Anthopleura elegantissima*. However, to date, there has been no validated set of reference genes for qRT-PCR described for reef building corals. The aim of this study presented here was to evaluate a set of candidate reference genes, using GeNorm and NormFinder, in reef-building corals exposed to chronic levels of elevated nitrogen followed by an acute increase in seawater temperature. These reference genes can possibly be used to normalize target genes in future gene expression studies targeting physiological stress in corals exposed to multiple stressors.

Materials and Methods

Coral collection and exposure to stressor(s)

Coral samples were collected from six randomly chosen colonies of *P. meandrina* at an approximate depth of 5 m from Leleiwi Beach Park in East Hawai`i. Colonies were split up into either of the two treatment tanks or into the control tank. The coral fragments were maintained in 75 L flow-through aerated aquaria set at a constant through flow rate of 7.15 ml s^{-1} and kept at 25˚ C. The tanks were placed under semi-permeable Duraweave material (Cover-all) that transmits light in the solar spectrum from 2500 -300 nm.

The colonies were allowed to acclimatize under the above conditions for a period of 3 weeks (Downs 2005). Then, the corals were placed in treatment tanks (control (C), +2º C sea water temperature (T), and nutrients and +2º C sea water temperature (NT)). Corals in the NT treatment were exposed to a constant addition of 20 μM NH_4 seawater for 32 days before beginning the acute increase in seawater temperature. After the 32 day exposure period to elevated levels of nutrients, both the NT and T treatments were subjected to a temperature increase in seawater over a continuous 48 h period. The water temperature of the aquariums was elevated +2° C to 27° C over a 3 h period and maintained above 27 °C. All treatments (C, T, NT) were sampled at 30 min, 6 h, 24 h, and 48 h after 27° C was reached. One coral fragment from each colony was collected per tank at each sampling time, wrapped in foil, and frozen immediately by immersion in liquid nitrogen. Once the tissue was frozen using liquid nitrogen, samples collected at each time period was stored for RNA analysis by submersion into RNA Later (Ambion), placed at 4° C for 24 h to allow the RNA Later to soak into the tissue, and then stored at -20° C to be used in qRT-PCR.

Selection of candidate reference genes

Five candidate reference genes were selected from the literature to be tested for their expression stability using mRNA from *P. meandrina*. Of the five genes, three had been frequently used as reference genes in gene expression studies of cnidarians primarily from class Anthozoa, and the remaining two were less frequently used reference genes. The three frequently used reference genes were: 18S rRNA, β-actin, and elongation factor (EF1α) (Yanze et al. 1999; Reynolds et al. 2000; Mitchelmore et al. 2002). The less frequently used reference genes examined in this study were senescence associated protein (PS) and Brn1 (Rodriguez-Lanetty et al. 2006; Rodriguez-Lanetty et al. 2007; Mayfield and Gates unpublished).

RNA isolation

Total RNA was isolated from *P. meandrina* using the Totally RNA kit (Ambion) largely following the manufacturer's protocol in an RNAse free environment. A few steps were modified from the protocol to reduce salt carryover from the RNA Later and reduce interference by Trizol found in the RNA

extraction kit. The tissue samples were placed directly on an RNAse free vacuum to remove excess RNA Later liquid then transferred to a sterile Whirl-pak 60 mL plastic sample bag (Nasco). 800 µL of the denaturation solution from the Totally RNA kit was pipetted on top of the coral fragment, and the coral tissue was air blown at 60 psi from the coral skeleton. The tissue slurry was added to a 2.0 mL tube filled with approximately 250 mg of sterile 0.5 mm diameter Zirconia/Silica beads (BioSpec Products) and vigorously shaken (Minibeadbeater-8 Cell Disrupter) for 1 min. The tube containing the tissue slurry was then centrifuged at 12,000 rpm for 2 min at 4 °C. A ¾ v. DEPC-treated water and an equal volume of Phenol:Chloroform:IAA (Promega) solution was added to the lysate and again vigorously vortexed with beads for 1 min. The remainder of the manufacturer's protocol was followed with an overnight precipitation of RNA, followed by a 30 min centrifugation period. The RNA pellet was resuspended with 19.5 µL of DEPC water and 0.5 µL of RNAsin ribonuclease inhibitor (Promega). RNA concentration in the final extracts was measured using the nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies). The RNA was further purified of genomic DNA contamination by DNAse I digestion, according to the manufacturer's protocol (Promega).

Reverse transcription and qPCR

A commercial reverse transcription kit was used according to the manufacturers instructions to reverse transcribe 1 µg of DNAse treated RNA in a 20 µL reaction (iScript cDNA Synthesis Kit, Bio-Rad). DEPC treated water was added to dilute the 20 µL RT reaction products to a final volume of 200 µL. The qPCR assays were performed by using a commercial 2X SYBR-Green master mix buffer (BioRad). qRT-PCR assay efficiency was calculated for each gene using a 6-step, 10-fold cDNA dilution series. Results were documented as cycle threshold values of background subtracted qPCR fluorescence kinetics.

Analysis of results

Relative expression quantities were calculated using the comparative C_T method from qPCR results (Vandesompele et al. 2002). Two gene stability analysis programs, GeNorm (Vandesompele et al. 2002) and NormFinder (Andersen et al. 2004), were used to rank reference genes in order of stability.

The GeNorm method, as previously described by Vandesompele et al. (2002), was used to determine the reference gene expression stability measures ("M-value"). The "M-value" for each gene is calculated as the arithmetic mean of pairwise variation between a particular gene and all other control genes. This program relies on the theory that the expression ratio for ideal internal control genes is identical in all samples, regardless of the experimental condition or treatment. Genes with low M values have the least variation in expression ratios with other genes and are the most stably expressed and vice-versa genes with a high M value have a large variation in the expression ratio are less stably expressed.

The second method of analyzing relative expression stability of candidate reference genes in this study was NormFinder, which has previously been described by Andersen et al. (2004). The relative expression of reference genes were analyzed for stability by NormFinder using GenEx software (Multid Analysis, California). NormFinder generates a gene stability measure using a "model based approach" by separately ranking a set of candidate reference genes according to their expression stability in a given sample set and given experimental design. The mathematical equation estimates the overall expression variation of each candidate reference gene in addition to the variation between sample subgroups of the sample and generates a "stability value" for each that is a direct measure for the estimated expression variation.

Results

Expression of reference genes during exposure to elevated heat and nitrogen levels

The raw expression levels for the five candidate reference genes that were analyzed fell into three categories based on transcript abundance: (1) high transcript abundance (average C_T value below 20): 18S; (2) median transcript abundance (average C_T value 20-25): β-actin, PS; and (3) low transcript abundance (average C_T value <25): EF1-alpha, Brn1.

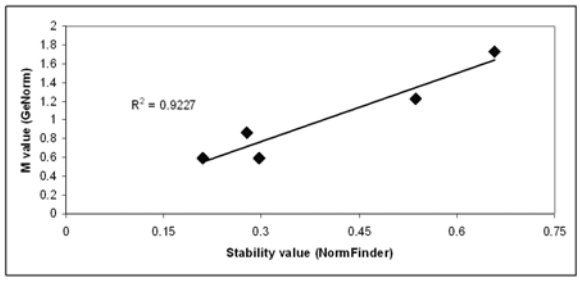

Figure 1: The correlation (R^2=0.9227, P=0.009) of expression values between reference gene ranking programs, M value (GeNorm, Vandesompele et al. 2002) and stability value (NormFinder, Andersen et al. 2004).

Analysis of gene-stability in reference genes

Both gene expression stability programs ranked the RNA targets similarly from most to least stable, with a strong correlation in raw stability values (R^2= 0.9227, P = 0.009) (Fig. 1). Results from both programs showed Brn1 was the most consistently expressed gene

followed by β-actin, and 18S (Fig. 1). Both programs also produced similar results with EF1α and senescence- associated protein (PS) exhibiting the least stable expression (Fig. 1). Because both expression stability programs had similar results, only GeNorm values are interpreted and were further used to analyze expression of *P. meandrina* samples. To ensure that the most stable genes were not co-regulated, each of the highest ranked genes was removed alternately, and gene stability rank was reassessed. No changes in the order of reference gene rank stability were observed. As shown in Fig. 2, the reference genes with the lowest M values and highest expression stability in *P. meandrina* coral tissue were Brn1 and β-actin with M values of 0.2095 and 0.2775 respectively. 18S was the third most stably expressed gene with an M value of 0.2963. These genes were designated to have stable expression because the M<0.4 (Vandesompele et al. 2002).

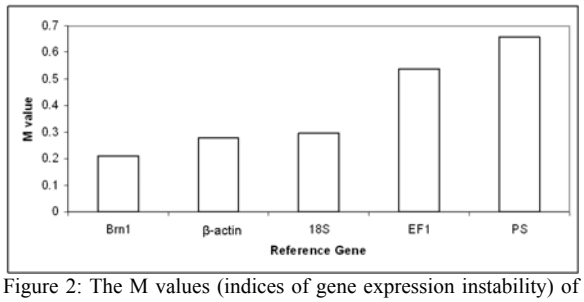

Figure 2: The M values (indices of gene expression instability) of candidate reference genes calculated from GeNorm analysis

Discussion

In this study, we sought suitable reference genes that could be used for future gene expression studies in the reef-building coral *P. meandrina* exposed to long term elevation of nitrogen followed by an acute increase in seawater temperature. Appropriate reference genes are necessary in gene expression studies to normalize sampling differences and identify the true expression differences in an experiment. In cases where no single normalization gene can be found it is best to use multiple genes to normalize data because the variation in multiple genes is less than the variation of a single gene (Andersen et al. 2004).

Several studies have compared different statistical programs to order candidate reference genes and found a similar ranking of stable reference genes within these programs (deBrouwer et al. 2006; Willems et al. 2006; Peltier and Latham 2008). Our study also compared the statistical programs GeNorm and NormFinder and found that candidate genes were ranked similarly by expression stability (Fig. 1).

The study described here shows that reference genes: Brn1, β-actin and 18S, are the most stably expressed in the coral, *P. meandrina*, under a long-term exposure to elevated nitrogen and an acute increase in sea water temperature (Fig. 2). A POU-III (acronym of Pit, Oct, Unc) class gene, Brn1, derived from a common eumetazoan ancestor, is expressed in the geosensory structures of vertebrates, insects and cnidarians (Erkman et al. 1996; Certel et al. 2000; Jacobs and Gates 2001; O'Brien and Degnan, 2002; O'Brien and Degnan 2003). The Brn1 gene is constitutively expressed at low levels in the cell most likely because of its conserved role in sensory cell development (O'Brien and Degnan 2002). The β-actin gene was the second most stably expressed gene in the set of reference genes (Fig. 2) and has been used as a reference gene for multiple cnidarian gene expression studies (Weis and Reynolds 1999; Reynolds et al. 2000; Rodriguez-Lanetty et al. 2007). β-actin encodes a ubiquitous cytoskeletal structural protein and is expressed at moderate levels in the cell. 18S rRNA, is in the small ribosomal subunit in most eukaryotes, and has been considered to be an ideal reference gene because rRNA is less likely to vary than mRNA under the same conditions (Bustin 2000). 18S rRNA as an internal control alone can be limiting because it may not always represent the overall cellular mRNA population (Suzuki et al. 2000). Although 18S ranked behind Brn1 and β-actin, this gene has shown to be constitutively expressed in many studies because rRNA synthesis is independent of mRNA synthesis (Franzellitti et al. 2005; Jorgensen et al. 2006; Tang et al. 2007).

Comparing the results of this study to previous qRT-PCR studies utilizing reference genes demonstrates the increased stability of a "new" reference gene, Brn1, which can possibly be used for stress experiments in reef-building corals alongside more popular reference genes like 18S and β-actin. Together, these reference genes can be used in future studies to normalize gene expression analysis of reef-building corals exposed to nutrient and temperature stressors.

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We would like to thank Drs. Mike Shintaku, Ruth Gates and Mauricio Rodriguez-Lanetty for their help in review of this manuscript. This project was funded by Hawaii EPSCoR Research Enhancement Activities Program (NSF EPS0556457) and University of Hawaii at Hilo Seed Money Grant to MT.

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CORAL HEALTH AND DISEASE ASSESSMENT IN THE U.S. PACIFIC TERRITORIES AND AFFILIATED STATES

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Abstract. Quantitative coral disease assessments were conducted at 40 different U.S. Pacific coral islands, banks, and atolls, spanning more than 5000 km apart and over a 40° latitudinal gradient Distribution and abundance of disease were determined based on 2, 25-m belt transects (2–6 wide) at 326 sites, and ten broad diseases were identified, affecting 25 scleractinian genera. Pacific-wide, the most geographically and taxonomically widespread diseases were bleaching and skeletal growth anomalies, detected at >65% islands/atolls and on 19 and 11 genera, respectively. Band diseases (black band and banded fungal disease) were rare (<0.1% prevalence), with only two cases enumerated on *Porites* and *Coscinaraea*. Although Pacific-wide mean prevalence was low, site-specific hotspots occurred at Johnston, Kure, and Rose Atolls, French Frigate Shoals, and Guam. Prevalence patterns also varied among coral taxa, with only a few families being disproportionately affected by disease: Poritidae, Acroporidae, and Faviidae. Of potential concern is the presence of white syndrome at Johnston Atoll and French Frigate Shoals which can result in severe and rapid tissue loss, particularly on the tabular *Acropora cytherea*.

Key words: Coral disease, prevalence, U.S.-affiliated Pacific Territories

Introduction

Disease is defined as any impairment that interferes with or modifies the performance of normal physiological functions, including responses to environmental factors, toxicants, and climate; infectious agents; inherent or congenital defects; or a combination of these factors (Wobeser 2006). Pacific-wide, there is growing concern pertaining to the threat of increased prevalence, geographic distribution, and host range of coral diseases, particularly in the Great Barrier Reef (GBR), the Marshall Islands, the Red Sea, the Philippines, East Africa, and the Hawaiian Archipelago (Willis et al. 2004; Aeby 2006; Harvell et al. 2007). NOAA's Coral Reef Ecosystem Division (CRED) has embarked on a long-term, broad-scale coral disease assessment and monitoring program aimed at documenting the distribution and prevalence of coral diseases on U.S. Pacific reefs and investigating the factors that may be contributing to the occurrence of disease. Within this framework, between 2006 and 2007, a total of 326 coral disease surveys were conducted at 5 major regions, including: main Hawaiian Islands, Northwestern Hawaiian Islands, American Samoa, the Pacific Remote Island Areas (PRIA), Guam, and the Commonwealth of the Northern Mariana Islands (CNMI). These surveys are a coordinated effort within NOAA's interdisciplinary, Pacific Reef Assessment and Monitoring Program (RAMP).

Materials and Methods

Disease assessments were conducted along two, haphazardly placed 25-m belt (2–6 m width) transects laid end to end, separated by approximately 5 m. The smaller survey area was implemented at disease-rich sites or where surge, currents, or sea conditions precluded expanded surveys. Within belt transects, all diseased coral colonies were enumerated, identified to genus level, measured in two planar dimensions, and assigned to 1 of 10 lesion categories including: skeletal growth anomalies (SGA), acute tissue loss (hereafter white syndrome; WSY), subacute tissue loss (hereafter tissue loss; TLS), pigmentation response (PRS), trematodiasis (TRE), endolithic fungal infection (EFI), banded fungal infection (BFI), black band disease (BBD), and 'other' (OTH), including algal and cyanophyte infections, and syndromes of unknown etiology (see NOAA 2009 for lesion characterization and description). Colonies exhibiting full, partial or spotty bleaching (BLE) were also tallied and thus, hereafter, bleaching is treated as a disease state. Case counts were complemented with digital photography, as well as tissue collections for future histological examination and verification. A second diver performed community structure surveys by counting and identifying, to the genus level, all colonies within 50–100 cm on both sides of each transect line. Alternatively, at 37 sites in the CNMI, colony counts were conducted using 15–16,

haphazardly placed 0.25 m^2 quadrats. All the above data allowed for the computation of prevalence per genus/disease state, as follows: P = [(total no. cases of a specific disease for the genus × 100) ÷ (colony density of that specific genus per site × total area surveyed for presence of disease)].

Results

A total of 1744 cases of disease were recorded at 67% (220) of the sites surveyed. Farallon de Pajaros in the northern CNMI was the only island where no disease was recorded. A summary of prevalence of disease for each island group and genera are presented in Table 1.

The assessments conducted in American Samoa revealed the highest archipelago-wide mean prevalence (4.3% SE 1.3). Island-specific mean prevalence was the greatest at Rose Atoll (11.7% SE 5.7) and the lowest at Swains Island (1.3% SE 0.8). Of the 62 sites visited, 38 (61%) contained disease, and 7 disease states were enumerated: BLE, SGA, WSY, BBD, PRS, EFI, and OTH. Archipelago-wide, diseases were observed on corals belonging to 14 genera, with *Montastrea*, *Favia*, and *Montipora* exhibiting the greatest percent of cases (30.7%, 34.4%, and 14.8%, respectively), and *Favia*, *Porites*, and *Coscinaraea* showing the greatest mean prevalence values (1.5%, 0.5%, and 0.5%, respectively). Other lesions (OTH), particularly algal/cyanophyte infections were the most numerous disease state, representing 75.3% of cases and notably abundant on 2 west-northwestern forereef sites at Rose Atoll, in the vicinity of a major shipwreck impact site (Schroeder et al. 2008). Island-wide mean prevalence of OTH at Rose amounted to 9.5 (SE 0.5) and affected corals in the genera *Favia*, *Montastrea*, *Porites*, and *Montipora*. In addition, BLE although present at all islands was sporadic and mild, with the highest prevalence values observed at Ofu-Olosega (8.5%); archipelago-wide, however, mean prevalence of BLE amounted only to 0.6% (SE 0.2). BLE occurred mostly on *Porites*, *Montastrea*, and *Montipora*. Lesions involving SGA were occasional, representing 9.4% cases and occurring predominantly around Tutuila and Ofu-Olosega on *Acropora* and *Astreopora*, respectively; archipelago-wide mean prevalence of SGA was 0.8% (SE 0.4). PRS and EFI were also sporadic (6.0% and 7% of cases, respectively), with the greatest island-specific mean prevalence values for Rose Atoll and Tau (1.9% and 3.4%, respectively). Finally, cases of WSY and BBD were rare, representing only 1.7% and 0.2% of total, respectively; archipelago-wide mean prevalence for these two disease states was also low, amounting to 0.06% (SE 0.03) and 0.01% (SE 0.01) respectively.

Of the 64 sites surveyed in the Northwestern Hawaiian Is., 81% contained disease, and 8 different types of lesions were identified: BLE, WSY, TLS, SGA, PRS, TRE, EFI, and OTH; with a region-wide mean prevalence of 3.1 (SE 0.6). Diseases were observed on the 4 main reef-building coral genera (*Porite*s, *Montipora*, *Acropora*, and *Pocillopora*), of which nearly 70% of cases occurred on *Porites*. The greatest number of disease states was recorded at French Frigate Shoals and the lowest at Laysan Island (8 and 2, respectively; Table 1). Mean disease prevalence was greatest at Kure Atoll (7.3% SE 3.7), while Necker Island exhibited the lowest (2.0% SE 1.8). Three disease states, PRS, BLE, and TRE, were the most widespread and abundant. Although BLE was quite common at Laysan and Lisianski, prevalence values were relatively low (1–5%); BLE was moderate and focal and mostly affected on corals of the genera *Porites* and *Montipora*. Lesions involving PRS were ubiquitous but also occurred at low levels of prevalence, not exceeding 3% at any of the sites visited. In addition, the parasitic *Porites* TRE occurred at moderate levels at most sites, except for 2 western backreef sites at Kure Atoll, where prevalence values reached 23–27%. This was the result of reduced numerical abundance of colonies of the host genus at the sites where cases of TRE were enumerated. Together with TRE, lesions involving TLS, BLE, and PRS were present at 7 of the 8 islands/atolls. The 2006 surveys encountered TLS lesions exclusively on colonies of the genus *Porites* and low island-wide mean prevalence values (< 0.5%). Finally, of potential concern are WSY conditions detected on the tabular *Acropora cytherea* only at French Frigate Shoals. Although prevalence values only amounted to 4% for any one site, French Frigate Shoals harbors the largest population of *A. cytherea* in the Hawaiian Archipelago.

For Guam and the CNMI, mean disease prevalence region-wide was relatively low (2.9% SE 0.4), but as in the other archipelagos, exhibited considerable spatial variability. Sixty-six sites at 12 different islands were surveyed in this region and a total of 8 disease states were recorded: BLE, SGA, WSY, TLS, PRS, EFI, BFI, and OTH. The highest island-wide prevalence occurred at Guam (7.3% SE 1.9) and the lowest at Pagan (0.1% SE 0.1). Disease affected the greatest number of scleractinian genera (19) in the Guam-CNMI region: *Porites*, *Astreopora*, and *Cyphastrea* accounted for more than 75% of all cases and exhibited the greatest prevalence. In addition, the 3 main disease states, BLE, EFI, and PRS (in order of prevalence) accounted for more than 65% of cases. Cases of BLE were common particularly around the inhabited Guam, Saipan, Tinian, and Rota and also at uninhabited Alamagan.

Site-specific prevalence of BLE was as high as 12% around Guam. However, mean archipelago-wide prevalence was only 1.3% (SE 0.4). For the most part, BLE was mild to moderate and affected primarily corals in the genus *Astreopora*. EFI cases were widespread and numerous, predominantly around Saipan on the genus *Cyphastrea*, where site-specific prevalence values were as high as 20%; archipelago-wide mean prevalence only reached 0.9% (SE 0.4). Lesions involving PRS on *Porites* occurred mainly at Guam and Tinian, where mean prevalence values amounted to 2.0% and 1.0%, respectively; archipelago-wide, mean prevalence was 0.5% (SE 0.2). Cases of WSY, BFI, TLS, and SGA were sporadic and occurred in low, region-wide mean prevalence (0.1–0.2%).

The 80 sites surveyed in the PRIA revealed a region-wide mean prevalence of 2.8% (SE 0.7), and 6 lesion types (SGA, WSY, TLS, BLE, PRS, OTH) were identified affecting 12 different scleractinian genera, with *Acropora*, *Montipora*, and *Porites* accounting for 92% of cases. Diseases occurred at 43 (54%) of sites surveyed, with SGA being the most geographically and taxonomically widespread disease, detected at nearly 40% of sites. SGA were particularly abundant at Johnston Atoll, where site-specific prevalence was as high as 25.3%. WSY, which was hosted by 4 scleractinian genera, including *Acropora*, *Montipora*, *Goniastrea*, and *Platygyra*, was the second most prevalent disease region-wide (mean prevalence = 0.9%, SE ± 0.1%) with nearly 90% cases occurring at Johnston Atoll. Additionally, PRS and OTH, particularly algal algal/cyanophyte infections and tube-worm infestations, were infrequent and occurred in low prevalences region-wide (range: 0.1 and 0.4%, respectively). Overall, mean prevalence of all diseases was the highest at Johnston Atoll (8.5% SE 2.5) and lowest at Baker Island (0.1% SE 0.1) and patterns of prevalence varied considerably among coral genera with *Acropora*, *Montipora*, and *Porites* being disproportionately affected by SGA and WSY. Of potential concern is WSY, which results in severe and rapid tissue loss, particularly on the tabular *Acropora cytherea* at Johnston Atoll.

Mean, region-wide prevalence amounted to 1.1% (SE 0.3) for the main Hawaiian Islands, where 54 sites were surveyed and 7 disease states enumerated: BLE, SGA, TLS, TRE, EFI, PRS, and OTH. Disease affected 6 coral genera, with *Porites* accounting for more than 90% of the total number of cases (316). Four lesion types, SGA, TLS, EFI, and PRS, were the most widespread and abundant; however, except for limited small areas of higher abundance, disease-specific mean prevalence amounted to less than 2%; All 7 diseases were recorded on corals around the inhabited islands of Hawaii and Maui; Kauai followed with 6 disease states. Limited insight is available for Oahu, as only 1 representative survey was completed in 2006. Kauai exhibited the greatest mean prevalence (3.7% SE 1.8) and, on surrounding reefs, EFI, SGA, and TRE were the most common lesions. The island with the second highest mean prevalence was Lanai (1.5% SE 1.3). This was a result of bleaching occurrence on a few colonies of *Leptastrea*; this genus exhibited overall low numerical abundance at the sites where bleached colonies were encountered. The more remote islands of Niihau and Lehua exhibited both the lowest number of disease states (2) as well as lowest mean prevalences (0.5% SE 0.3 and 0.2% SE 0.1, respectively).

Discussion

This study represents perhaps the most extensive quantitative survey of coral disease across the U.S. Pacific reefs. Surveys detected disease at 67% of sites, and patterns of distribution and abundance varied considerably within and among islands, regions, and coral genera, with only a few taxa being disproportionately targeted by disease. The Northwestern Hawaiian Islands exhibited the greatest percent occurrence of disease with nearly twice as many cases (35%) as any other region; the PRIA exhibited the lowest (9.8%). Additionally, the most numerically abundant disease states Pacific-wide were PRS (20.5%) and BLE (16.1%); and the least abundant, BBD and BFI (0.05% each). However, relative to coral colony density, the most prevalent diseases were BLE and SGA (0.64% and 0.58%, respectively). Pacific-wide, mean prevalence was relatively low (2.8% SE 0.5), with the lowest mean value registered for the main Hawaiian Islands (1.1% SE 0.3) and the highest for American Samoa (4.3% SE 1.3). These archipelago-wide means are lower than those in disease-stricken regions of the Great Barrier Reef and the Caribbean (Puerto Rico, Mexico, and Jamaica; range 6.3–16.6%; Weil 2004; Willis et al. 2004). However, island-specific mean prevalences as high as 7.3%, 8.3%, and 11.7% for Guam, Johnston, and Rose Atoll, respectively, underpin the importance of continued coral disease monitoring to provide warning regarding the potential risk of specific coral populations.

Disease distribution also varied among island groups. For example, the parasitic TRE on *Porites* was only recorded in the Hawaiian Archipelago, while BBD and BFI were exclusive to American Samoa and the CNMI, respectively. The other disease states, BLE, PRS, SGA, WSY, TLS, EFI, and OTH, were more widely distributed among island groups. Additionally, different island groups exhibited differing levels of abundance and prevalence of

specific lesions. For example, BLE conditions were the most prevalent disease state in the Northwestern Hawaiian Islands and Guam-CNMI. Conversely, SGA and WSY were the most prevalent diseases in the PRIA, while OTH was in American Samoa. Factors including, but not limited to pathogen distribution and life history, environmental conditions and disturbances, host abundance, susceptibility, disease transmissibility and virulence may determine the occurrence and prevalence of disease (Wobeser 2006).

The 1744 cases tallied in this study occurred on 25 different coral genera, belonging to 10 scleractinian families. The Poritidae exhibited both the highest frequency of occurrence and mean prevalence in the main Hawaiian Islands (90.1% of cases, mean prevalence = 0.6%) and the Northwestern Hawaiian Islands (69.8% of cases, mean prevalence = 2.4%, respectively). Within the Poritidae, TRE and PRS were the most numerically abundant diseases (60% of cases, combined), while BFI and WSY were the only two syndromes not observed on this taxon. Comparatively, the Acroporidae was the taxon most susceptible to disease in the PRIA (65% of cases, mean prevalence = 1.3%) and the Faviidae in American Samoa (58.2% of cases, mean prevalence = 2.2%). Pacific-wide, SGA and WSY were the most numerically abundant and prevalent diseases on the Acroporidae (52% and 0.6%, respectively), while OTH (particularly algal/cyanophyte infections) were the most numerically abundant and prevalent lesions on the Faviidae (62% and 0.7%, respectively).

Overall, the three major families, Poritidae, Acroporidae, and Faviidae, hosted the greatest number of disease states (8, 6, and 6, respectively), as well as the greatest levels of mean prevalence (1.0%, 0.9%, and 0.7%, respectively). Pacific-wide, these three families are amongst the most numerically abundant and diverse taxa, representing a key community structural component. For example, these 3 families accounted for 70–80% of the live coral cover and 65–85% of colonies on the study reefs in American Samoa, the PRIA, and Guam-CNMI. Similarly, in the Hawaiian Archipelago, *Porites* alone represents more than 60% of the live coral cover and more than 47% of colonies (Vargas-Ángel and Kenyon unpubl. data). Recent studies indicate an escalating number of diseases and prevalence levels affecting a selected number of scleractinian taxa, whereby *Acropora*, *Montastraea*, and *Colpophyllia* appear highly susceptible to disease on Caribbean reefs, while *Porites*, *Acropora*, *Montipora* and *Pocillopora* are the chief disease-hosting genera on Indo-Pacific reefs (Weil 2004; Willis et al. 2004; Aeby 2006; Harvell et al. 2007). To what extent host susceptibility to disease is determined by numerical abundance and/or a life history trait is an important topic for further scientific investigation.

This study represents the first comprehensive attempt at assessing the distribution and abundance of coral diseases in the U.S.-affiliated Pacific States and Territories. Although archipelago-wide mean prevalences are relatively low (1.1–4.3%; Table 1), site-specific hotspots occur at Johnston, Kure, and Rose Atolls, French Frigate Shoals, and Guam. In addition, the scleractinian families Poritidae, Acroporidae, and Faviidae appear to be disproportionately affected by disease. Potential outbreaks of host-specific diseases can be a source of concern, given that these families are key contributors to reef building and structural dynamics in many U.S. Pacific reefs. Periodic regional monitoring, coupled with continued targeted research, will allow a better understanding of the natural coral disease dynamics and, therefore, help managers formulate informed decisions regarding the potential risk of specific coral populations.

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Table 1. Mean prevalence (SE) of coral disease at 40 islands, banks, and atolls in the US-affiliated Pacific States and Territories. BLE: bleaching, SGA: skeletal growth anomalies, WSY: acute tissue loss or white syndrome, TLS: subacute tissue loss, TRE: trematodiasis; PRS: pigmentation response; EFI: endolithic fungal infections, BFI: banded fungal infections, BBD: black band disease, OTH: other syndromes including algal and cyanophyte infections, parasite infestations, and other lesions of unknown etiology, $\overline{X}$: island-wide mean prevalence computed based on all survey sites. Region: Main Hawaiian Islands (MHI), Northwestern Hawaiian Islands (NWHI), American Samoa (AMSA), the Pacific Remote Island Areas (PRIA), and Guam and the Commonwealth of the Northern Mariana Islands (CNMI). FFS = French Frigate Shoals; PHR = Pearl and Hermes Atoll. Taxa affected by disease are listed in order of decreasing prevalence.

Region	BLE	SGA	WSY	TLS	TRE	PRS	EFI	BFI	BBD	OTH	$\overline{X}$	Taxa affected
MHI												
Hawaii	0.0	0.1	0.0	0.1	0.0	0.4	0.0	0.0	0.0	0.0	0.7	
Maui	0.0	0.2	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.6	
Kauai	0.2	0.8	0.0	0.2	0.7	0.0	1.6	0.0	0.0	0.2	3.8	*Porites*, *Montipora*, *Pocillopora*, *Psammocora*, *Pavona*
Lanai	1.1	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	1.5	
Molokai	0.0	0.1	0.0	0.1	0.0	0.3	0.1	0.0	0.0	0.0	0.6	
Niihau	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.1	0.5	
Lehua	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	
Mean	**0.2**	**0.2**	**0.0**	**0.0**	**0.2**	**0.2**	**0.3**	**0.0**	**0.0**	**0.0**	**1.1% (SE 0.3)**	
NWHI												
Necker	1.0	0.0	0.0	0.1	0.4	0.5	0.0	0.0	0.0	0.2	2.0	
FFS	1.5	0.2	0.5	0.1	0.2	0.8	0.1	0.0	0.0	0.3	3.4	
Maro	1.2	0.0	0.0	0.1	0.2	0.8	0.0	0.0	0.0	0.0	2.3	
Laysan	2.9	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	3.3	*Porites*, *Montipora*, *Acropora*, *Pocillopora*
Lisianski	2.5	0.1	0.0	0.4	0.3	0.3	0.0	0.0	0.0	0.1	3.6	
PHR	0.1	0.0	0.0	0.1	0.3	0.4	0.0	0.0	0.0	0.7	1.0	
Midway	0.0	0.0	0.0	0.4	0.0	0.1	0.0	0.0	0.0	0.3	0.6	
Kure	0.0	0.0	0.0	0.1	7.0	0.0	0.2	0.0	0.0	0.1	7.3	
Mean	**1.0**	**0.0**	**0.1**	**0.2**	**1.2**	**0.4**	**0.1**	**0.0**	**0.0**	**0.3**	**3.1% (SE 0.6)**	
AMSA												
Tutuila	0.3	1.6	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.9	3.0	*Favia*, *Porites*, *Coscinaraea*,, *Acropora*, *Montastrea*, *Astreopora*, *Favites*, *Montipora*, *Leptoria*, *Platygyra*, *Fungia*, *Pavona*, *Pocillopora*, *Goniastrea*
Ofu-Olo	1.9	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	
Ta'u	0.3	0.4	0.0	0.0	0.0	0.0	3.4	0.0	0.0	0.0	4.1	
Swains	0.8	0.5	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	1.3	
Rose	0.1	0.0	0.1	0.0	0.0	1.9	0.0	0.0	0.0	9.6	11.7	
Mean	**0.6**	**0.8**	**0.1**	**0.0**	**0.0**	**0.0**	**0.5**	**0.0**	**0.1**	**2.2**	**4.3% (SE 1.3)**	
PRIA												
Wake	0.2	3.2	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	3.9	
Johnston	0.0	3.4	3.6	0.0	0.0	1.4	0.0	0.0	0.0	0.1	8.5	*Acropora*, *Porites*, *Pocillopora*, *Montipora*, *Gardineroseris*, *Astreopora*, *Platygyra*, *Favites*, *Goniastrea*, *Hydnophora*, *Leptastrea*, *Favia*
Baker	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	
Howland	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	
Jarvis	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.3	
Palmyra	1.0	0.3	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	1.4	
Kingman	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	
Mean	**0.2**	**1.4**	**0.9**	**0.0**	**0.0**	**0.4**	**0.0**	**0.0**	**0.0**	**0.1**	**2.8% (SE 0.7)**	
Guam-CNMI												
Guam	4.2	0.1	0.2	0.5	0.0	2.0	0.2	0.1	0.0	0.0	7.3	
Rota	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	
Tinian	0.4	0.1	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	1.3	
Saipan	0.8	0.0	0.0	0.0	0.0	0.8	2.6	0.0	0.0	0.1	4.4	*Porites*, *Astreopora*, *Cyphastrea*, *Platygyra*, *Gardineroseris*, *Montipora*, *Echinopora*, *Goniastrea*, *Coscinaraea*, *Acropora*, *Favia*, *Psammocora*, *Plesiastrea*, *Hydnophora*, *Pavona*, *Goniopora*, *Pocillopora*, *Turbinaria*
Sarigan	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.3	0.4	
Guguan	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	
Alamagan	4.3	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.4	5.0	
Pagan	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	
Agrijan	0.1	0.0	2.2	0.0	0.0	0.0	0.0	1.4	0.0	0.0	3.8	
Aguijan	0.0	0.0	11.1	0.0	0.0	0.0	0.0	0.0	0.0	5.6	16.7	
Asuncion	0.0	0.3	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.7	
Maug	0.5	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.7	
Farallon de Pajaros	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Mean	**1.3**	**0.2**	**0.2**	**0.1**	**0.0**	**0.5**	**0.4**	**0.1**	**0.0**	**0.2**	**2.9% (SE 0.4)**	

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White band syndromes in *Acropora cervicornis* off Broward County, Florida: Transmissibility and rates of skeletal extension and tissue loss

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Abstract. The high latitude thickets of *Acropora cervicornis* off Broward County flourish despite the presence of natural and anthropogenic impacts. These populations provide a unique study opportunity which stands out against the disease stricken areas of the Florida Keys. This study uses time sequenced photographs to examine how *A. cervicornis* is coping with white band syndrome stressors. Variables monitored include healthy colony skeletal extension rates, diseased colony skeletal extension rates, and tissue loss. The transmissibility of the white band syndromes was examined through tissue grafting experiments. Skeletal extension rates of healthy and diseased fragments averaged 0.94 ± 0.4 cm mo^{-1} and 0.79 ± 0.4 cm mo^{-1} respectively. Mean linear tissue loss from disease signs was 1.2 ± 2.5 mm d^{-1} or 3.7 ± 7.5 cm mo^{-1}. In transmission experiments, 72.5% of all trials involving direct tissue contact resulted in low or no disease sign transmission. *A. cervicornis* thickets in Broward County are growing similar to other studies in Florida but faster than other areas of the Western Atlantic while tissue loss from disease is lower. White band syndromes are always present in Broward County, but the low prevalence and transmission of the syndrome seems to limit its affect on the thickets.

Key words: White band disease, *Acropora cervicornis*, growth rates, tissue loss, disease transmission

Introduction

Surveys in Broward County, FL have discovered several thickets of *Acropora cervicornis* (Thomas et al. 2000, Vargas-Angel et al. 2003). Despite the presence of abundant natural and anthropogenic impacts, the *A. cervicornis* thickets are exceptionally healthy. These thickets provide a unique site for studying white band syndrome effects on acroporid populations when compared to the disease-stricken populations of the majority of the Caribbean including the Florida Keys National Marine Sanctuary (Vargas-Angel et al. 2003).

Recent observation of *A. cervicornis* populations directly off Fort Lauderdale, Lauderdale-By-The-Sea, and Pompano beaches in Broward County and other locations have shown that white band disease seems more complex than previously believed. For this reason the term white band syndromes is being used to include all cases. Three patterns of tissue loss have been observed during this study. The first is a fast moving, clearly defined white band of tissue necrosis next to 3-7 cm of denuded skeleton followed by algal successional stages. The second is similar to the first but it is a slower moving, well defined white band of denuded skeleton, followed by no more than 1-2 cm of denuded skeleton, then algae. Both of these tissue loss patterns resemble previously described patterns referred to as white band Type I (Gladfelter 1982, Peters 1984). White band type II has been reported in the Bahamas with an area of bleached tissue preceding the necrotic tissue (Ritchie and Smith 1998). White band type II was not seen during this study. The third observed tissue loss pattern looks similar to White Pox observed on *Acropora palmata*. This condition has irregular areas of denuded skeleton, termed 'patchy necrosis,' which spreads rapidly, regularly killing large portions of colonies. Similar patterns of rapid tissue loss were reported around the time when the first observations of white band disease occurred (Bak and Criens 1981). *Serratia marcescens* has been found to be a cause of similar looking patchy necrosis in *A. palmata* (Patterson et al. 2002). Williams and Miller discuss similar tissue loss patterns in their study at White Bank off Key Largo and refer to it as 'rapid tissue loss' (Williams and Miller 2005). It is still unclear if or how this rapid tissue loss is linked to the white band diseases. The presence of both in Broward's *A. cervicornis* thickets provides a unique opportunity for study.

Many studies including Atlantic and Gulf Rapid Reef Assessment surveys show that disease continues to be a considerable stressor on acroporid populations in the western Atlantic (Lang 2003, Sutherland et al. 2004, Weil 2004, Weil et al. 2006). Disease, low sexual recruitment, and anthropogenic impacts are to cause for the Caribbean acroporid corals to be listed

as threatened on the Endangered Species Act (Anonymous 2005, Hogarth 2006) and listed as critically endangered under the International Union for the Conservation of Nature (IUCN) Red List criteria (Carpenter et al. 2008). This project was developed as part of a larger project investigating *A. cervicornis* thickets off Broward County, Florida for comparison to other western Atlantic acroporid populations. The primary goals of this project were to ascertain 1) healthy and diseased colony growth rates recorded in terms of linear skeletal extension not only for comparison to other populations but also to determine if there was a colony-wide influence from white band syndromes, 2) rates of tissue loss caused by white band syndromes recorded in terms of linear tissue loss, and 3) incidence of disease sign transmission from in-situ tissue grafting experiments confirmed via visual inspection.

Material and Methods

1) Skeletal Extension Rates – Colonies of *A. cervicornis* demonstrating no signs of white band syndromes and those with disease signs were identified for monitoring. On those colonies, branches with healthy tissue were marked 5-7 cm below the axial polyp with cable tied tags. Measurements from cable tie to branch tip were determined through photos calibrated with Coral Point Count with Excel extensions (CPCe) using the ruler photographed next to the branch (Kohler and Gill, 2006). All experiments were conducted at two different thickets concurrently from July through September 2007 and November 2007 through February 2008. Each site had 10 healthy and 10 diseased colonies tagged giving a total of 40 healthy fragments monitored and 40 diseased fragments monitored.

2) Tissue Loss Rates – Cable ties were placed near the tissue/skeleton interface on diseased colonies and used as a baseline from which progression of the disease front could be measured using CPCe in the same method described above. Linear movement of the tissue/skeleton interface was measured by calculating the difference between the cable tie and live tissue between measurements. This was monitored with the same method as above totaling 40 fragments.

To establish that the calibrated photo method is comparable to using calipers in-situ and an acceptable method for determining measurements, a small test was conducted. Five branches of *A. cervicornis* were tagged as they were in the experiment. Measurements were calculated using both methods. Caliper measurements were taken five times rotating around the axis of the branch. Photos were taken from five different angles. A two-sample paired t-Test was used to compare the two methods for each branch measured.

3) Transmissibility – Transmission of white band syndrome signs was observed with direct tissue contact via grafting experiments. There were four different trials 1) a diseased branch with a portion of an active lesion attached to a healthy colony, 2) a healthy branch attached to an active lesion on a diseased colony, 3) a healthy branch from a colony exhibiting disease signs attached to a healthy colony, and 4) a healthy branch from a healthy colony attached to a healthy colony. Branches were simply attached with cable tied tags and observed for disease sign transmission. Ten branches were used for each trial at each site giving a total of 160 fragments. Transmissibility was monitored through observation and photos. Each photographed tissue graft was assigned a classification based on the extent of transmission. The possibilities of transmission are outlined below (Fig. 1).

Classification	Explanation
None	No observed change in tissue color, integrity, or mucus output and no tissue loss on the 'healthy branch'.
Low	Margin of tissue contact showing whitening and possibly some tissue necrosis.
Moderate	'Healthy branch' clearly exhibiting tissue necrosis along grafting boundary.
Severe	Considerable tissue loss due to necrosis and most likely mortality of the transplanted fragment and host portion of the colony.

Figure 1: Chart outlining the four possible outcomes of a transmissibility experiment.

The following photos (Fig. 2-5) are examples of the possible transmission classifications.

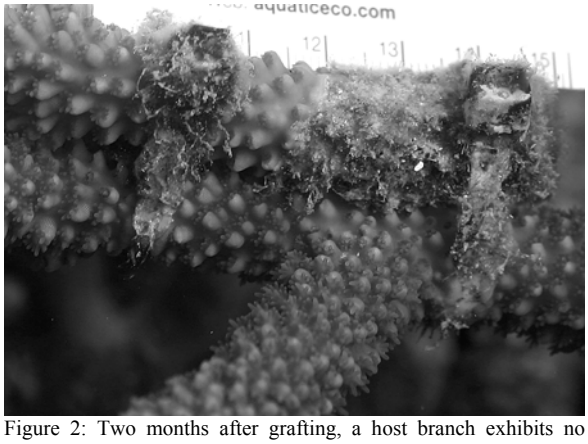

Figure 2: Two months after grafting, a host branch exhibits no transmission while the white band on the transplanted branch has ceased moving and the exposed skeleton was colonized by algae.

Figure 3: Nine days after grafting, a host branch exhibits low transmission while the transplant still has some healthy tissue.

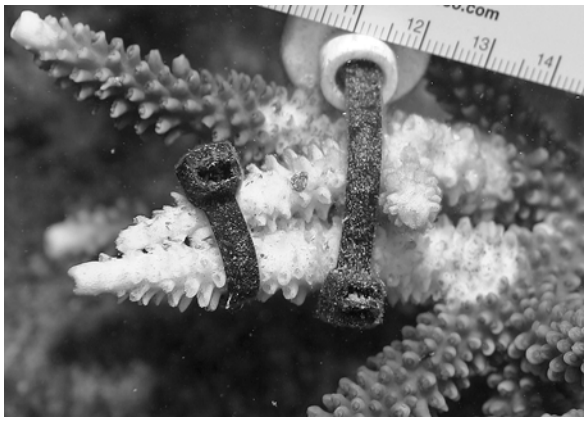

Figure 4: Nine days after grafting, a host branch is demonstrating moderate transmission while the transplant has lost almost all tissue.

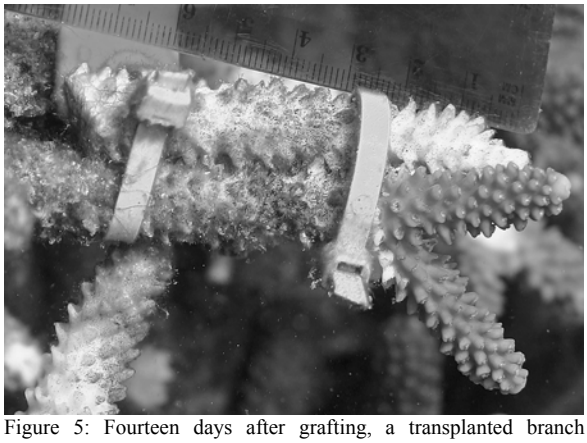

Figure 5: Fourteen days after grafting, a transplanted branch demonstrating severe transmission with no live tissue and the host branch is left with a small amount of tissue on the tip.

Results

The following chart (Fig. 6) lists the rates of healthy and diseased skeletal extension and tissue loss recorded during this experiment.

Measurement	Calculated Means
Healthy Skeletal Extension Rates	0.94 ± 0.4 cm mo^{-1}.
Diseased Skeletal Extension Rates	0.79 ± 0.4 cm mo^{-1}
Diseased Tissue Loss (linear)	1.2 ± 2.5 mm d^{-1} or 3.7 ± 7.5 cm mo^{-1}

Figure 6: Measurements reported as (mean ± SD).

A two sample paired t-test to compare healthy and diseased colony growth rates was conducted, with $\alpha=0.05$, $p=0.37$. This leads to the conclusion that there is no difference between the two skeletal extension rate means.

The comparison of five calibrated photos and five caliper measurements each of five different branches was analyzed using a paired t-test for sample means. With $\alpha=0.05$, the resulting p-values were 0.98, 0.82, 0.31, 0.61, and 0.64. All tests conclude that there is no difference between the two measurement means.

The following charts (Fig. 7-10) list the classifications of tissue grafting experiments in terms of percentage at each location at the termination of each experiment. The summer run was July through September. The winter run was November through February.

Diseased X Healthy				
Classification:	None	Low	Moderate	Severe
Scooter (S)	70%	10%	0%	20%
Oakland (S)	10%	0%	0%	90%
Scooter (W)	30%	10%	30%	30%
Oakland (W)	30%	60%	10%	0%

Figure 7: Transmission classifications for diseased branches attached to a healthy colony host. (S) = Summer (W) = Winter.

Healthy X Diseased				
Classification:	None	Low	Moderate	Severe
Scooter (S)	10%	0%	10%	80%
Oakland (S)	0%	0%	0%	100%
Scooter (W)	0%	10%	30%	60%
Oakland (W)	30%	20%	10%	40%

Figure 8: Transmission classifications for healthy branches attached to a diseased colony host.

Healthy from Diseased X Healthy				
Classification:	None	Low	Moderate	Severe
Scooter (S)	90%	10%	0%	0%
Oakland (S)	100%	0%	0%	0%
Scooter (W)	100%	0%	0%	0%
Oakland (W)	90%	0%	10%	0%

Figure 9: Transmission classifications for a healthy branch from a colony exhibiting disease signs attached to a healthy colony host.

Healthy from Healthy X Healthy				
Classification:	None	Low	Moderate	Severe
Scooter (S)	80%	0%	0%	20%
Oakland (S)	100%	0%	0%	0%
Scooter (W)	100%	0%	0%	0%
Oakland (W)	100%	0%	0%	0%

Figure 10: Transmission classifications for a healthy branch from a healthy colony attached to a healthy host.

Discussion

The table below (Fig. 11) lists annual growth rates of *A. cervicornis* recorded in several locations in the western Atlantic.

Growth Rate (cm y^{-1})	Location	Source
4	Dry Tortugas	(Vaughn 1915)
10.9	Key Largo, FL	(Shinn 1966)
11.5	Eastern Sambo, FL	(Japp 1974)
10	Key Largo, Florida	(Shinn 1976)
7.1	U.S. Virgin Islands	(Gladfelter et al. 1978)
3 to 4	Exuma, Bahamas	(Becker and Miller 2001)

Figure 11: Adapted from Atlantic Acropora Status Review (*Acropora* Biological Review Team 2005).

After converting this study's skeletal extension rates, healthy colonies have an average growth rate of 11.3 cm yr^{-1} and diseased colonies have an average growth rate of 9.5 cm yr^{-1}. These rates are within the same range of other Floridian *A. cervicornis* populations. Analyzing the data with a two sample paired t-test concludes that there is no statistically significant difference between the skeletal extension rate means of healthy or diseased colonies. This demonstrates that white band syndromes only impact the health of the coral at the location of the lesions and does not affect the rest of the colony's growth.

Not many studies have reported disease progression in terms of linear tissue loss. Williams and Miller (2005) discuss patterns of tissue loss similar to those observed in this study. This method of tissue loss termed 'rapid tissue loss' was seen during the study however, every effort was made to select corals that did not demonstrate rapid tissue loss. Corals that exhibited tissue loss patterns conforming to previous descriptions of white band syndrome type I were selected for this study. This study found mean tissue loss to be 0.12 ± 0.25 cm d^{-1}. Williams and Miller observed rates of 4 cm d^{-1} (Williams and Miller 2005) while other studies reported maximum rates of 2 cm d^{-1} (Antonius 1981, Gladfelter 1982, Peters et al. 1983). It seems that other factors must play a role in tissue loss rates such as temperature, season, nutrients, feeding scars, and recent branch breakage.

Transmission experiments involved four different tests. When a diseased branch was attached to a healthy colony the results were mixed. One site had 90% severe transmission. This ended up being an odd response. In all other treatments, the majority of transmission was not severe. It appears that some colonies may have the ability to resist transmission of the disease. Certain genotypes have been proven to be resistant to white band syndromes (Vollmer and Kline 2008). However, since there was some transmission, it appears there must be some sort of tranfer of causative agent or pathogen.

When a small piece of a healthy branch was attached to the lesion of a diseased colony the results were consistent. The majority of the transplants had severe transmission. There were some branches that did not transfer the disease signs (10% in the summer at one site and 30% in the winter at the other site). This supports studies that have shown that injuries such as a feeding scar from corallivorous snails or a fireworm (*Hermodice carunculata*) or in this case branch breakage can be a precursor to disease (Williams and Miller 2005, Sussman et al. 2003).

When a healthy branch from a diseased colony was transplanted to a healthy host the results were consistent. 90-100% of the trials showed no transmission of disease signs. This implies that a pathogen or causative agent is most likely present on healthy portions of a colony but are more concentrated on areas of active tissue loss caused by the white band syndromes.

When a healthy branch from a healthy colony was transplanted to a healthy colony the results were consistent. 100% no transmission was observed in all trials except one which had 20% severe. This further supports the idea from the previous paragraph that a causative agent is present on healthy portions of the colony.

During the transmission experiments, despite the effort to choose only those branches exhibiting disease signs resembling white band disease type I, when a transmission experiment lead to a transfer of disease signs, some branches demonstrated disease signs of white band type I and others the rapid tissue loss discussed earlier. Although each transmission experiment had differing results, 72.5% of all the tissue grafts had low or no transfer of disease signs.

When comparing methods using calipers or calibrated photos for measuring a branch of *A. cervicornis*, all paired t-tests for sample means resulted in p-values that conclude there is no statistically significant difference in the two methods means. The photo method was chosen to be less invasive. The only contact with the coral is the installation of the tag. If the tag is connected firmly with a cable tie gun, it will prevent tag movement and tissue irritation, the fragments can still thrive. Several tagged fragments started to overgrow their tags in just a few months.

In summary, the skeletal extension rates in Broward County are similar to previously recorded rates in Florida. These rates however, seem to be significantly higher than other areas of the western Atlantic. Tissue loss rates due to white band syndromes are faster than corals are growing but less than previously reported rates from the Florida Keys and other locations.

Transmissibility of white band syndromes appears to be more severe when involving small fragments but transmission is not always severe, supporting studies that have shown that tissue damage can be a precursor to disease. A significant portion of tissue grafts did not demonstrate disease signs transfer when directly attached to a diseased colony supporting other findings that colonies can be resistant. Future research such as genotyping of Broward's thickets could prove interesting regarding colony resistance. Using the calibrated photo method will allow future studies to make consecutive measurements with minimal interference with the normal growth and health of *A. cervicornis* colonies. When compared to other western Atlantic populations this study demonstrates that the *A. cervicornis* thickets in Broward County are healthy and growing faster. Despite the constant presence of white band syndromes, the low incidence and low transmission of disease signs seems to play a significant role in the good health of these populations. There is still much to be understood of the white band syndromes and the thickets of Broward County provide an excellent study site for such research.

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Ultrastructural and histological analysis of Dark Spot Syndrome in *Siderastrea siderea* and *Agaricia agaricites*

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Abstract. Dark Spot Syndrome (DSS) typically manifests in scleractinian corals as lesions of varying color, size, shape and location that can result in skeletal changes and tissue death. A causative agent for DSS has not yet been identified. The objective of this study was histological and ultrastructural comparison of the cellular and skeletal characteristics of DSS-affected and healthy *Siderastrea siderea* and *Agaricia agaricites*. The greater resolution possible with transmission electron microscopy (TEM) revealed microbial activity and tissue changes not resolvable utilizing histology. DSS-affected tissue had less integrity, with increasing cellular degradation and vacuolization. A high concentration of electron dense inclusions, which appear to be zymogen granules, was concentrated in the calicodermis and adjacent gastrodermal layer. Numerous endolithic fungal cells were observed directly adjacent to the calicodermis in DSS-affected *S. siderea*. Numerous unidentified endolithic cells were observed directly adjacent to the calicodermis in DSS-affected *A. agaricites*. These observations suggest that the coral may be using a digestive enzyme as a defensive mechanism against endolithic cellular invasion.

Key words: Dark Spot Syndrome, *Siderastrea siderea*, *Agaricia agaricites*, ultrastructure, TEM.

Introduction

A significant increase in the incidence and prevalence of coral diseases and the number of species affected has been observed worldwide and likely plays a major role in the decline of reef-building species (Rosenberg & Loya 2004). Most coral diseases are not well characterized, and effects of these diseases on corals at the cellular level have yet to be fully elucidated. Thus, the use of multiple approaches, including molecular, ultrastructural and histological techniques is needed in the investigation of coral disease and pathology.

Siderastrea siderea is an important scleractinian reef-building coral, commonly found throughout south Florida and the Caribbean. Of the several species of Caribbean corals affected by Dark Spot Syndrome (DSS), *S. siderea* is most frequently affected (Gil-Agudelo et al. 2004, Weil 2004, Borger 2005, Gochfeld et al. 2006). DSS typically manifests as black, brown or purple lesions of varying size and shape that can occur across the coral colony surface and at colony edges. DSS does not always cause tissue death; the lesions may disappear without any evident damage, or they can resolve and reappear on a different area of the colony (Gochfeld et al. 2006). If lesions are persistent, development of a depression in the skeleton or tissue necrosis is possible. Even if there is no tissue loss, underlying skeletal changes may occur. These changes may include a permanent dark skeletal discoloration that is observable in longitudinal skeletal slabs (Renegar pers obs.). To date, the cause of DSS has yet to be identified.

Unlike *S. siderea*, which is affected by DSS throughout its range, *Agaricia agaricites* appears to be affected by DSS in certain locations (e.g., Colombia: Garzón-Ferreira et al. 2001; Cayman Islands: Coelho & Manfrino 2007), but not others (e.g., Bahamas: Gochfeld, pers. obs.). DSS in *A. agaricites* typically manifests as much darker brown or black patches than on *S. siderea*. It is not yet known whether DSS found on these species has a common cause or similar impacts at the cellular level. The objective of this study was to use a combination of ultrastructural and histological techniques to describe cellular characteristics and possible pathogenic microbes in DSS-affected and healthy *S. siderea* and *A. agaricites*.

Materials and Methods

Tissue samples of *S. siderea* were collected from 10 healthy and 9 diseased colonies at Lee Stocking Island, Bahamas in July 2006, and from 2 healthy and

6 diseased colonies at Little Cayman, Cayman Islands in June 2007. Samples of 4 healthy and 4 diseased colonies of *A. agaricites* were collected from Little Cayman in June 2007. Examination of the *S. siderea* samples did not indicate substantial differences among locations or years. A fragment of each healthy colony and two fragments of each diseased colony (healthy and DSS-affected tissues) were fixed in glutaraldehyde fixative solution [2 mL 70% glutaraldehyde in 68 mL cacodylic buffer (2.16 g cacodylic acid in 200 mL of 0.22 µm filtered seawater)] in the field. Samples were maintained at 4°C in the fixative for 4-6 days, then divided into three subsets: two for TEM (calcified and decalcified) and one for histological analysis.

For TEM analysis, pieces of tissue approximately 2 mm^2 in size were removed from each fixed colony and post-fixed in buffered 1% osmium tetroxide solution (5 mL 4% aqueous osmium tetroxide in 30 mL of cacodylic buffer) for 1 hour. Samples were then rinsed in buffer, and the sample subset to be decalcified was placed in a diluted decalcification solution (0.032 g potassium sodium tartrate, 0.56 g sodium tartrate, 2.8 g EDTA, 400 mL 12 N HCl in 33.6 L dH_2O). The solution was changed frequently (every 1-2 hours) until each sample was completely decalcified. Samples were dehydrated in a graded series of ethanols, embedded in Spurr resin, and ultrathin sections were cut (40 to 60 nm thick) using a Sorval MT-2 ultramicrotome fitted with a diamond knife. Sections were retrieved on nitrocellulose and carbon coated 200 mesh copper grids, stained with Reynolds lead citrate and/or 2% uranyl acetate solution, and viewed in a Phillips 300 TEM. Electron dispersive spectroscopy (EDS) was used to evaluate elemental composition. Histological samples were decalcified in a 5% HCl/EDTA solution, dehydrated and embedded in Paraplast®, sectioned at 4 µm, and stained with Movat's Modified Pentachrome or Hematoxylin & Eosin.

As mucocyte size has been demonstrated to increase under stress, (Vargas-Ańgel et al. 2007), a quantitative comparison of mucocyte size in *S. siderea* was made from sections that were 500 µm from the aboral interface in 7 healthy and 4 DSS-affected colonies from the Bahamas. The length and width of mucocytes were determined in 5 random fields of view from each section (1000X magnification). Healthy tissue on DSS-affected colonies was taken from at least 1 cm away from DSS-affected tissue. Paired t-tests were used to compare mean cell sizes between healthy and DSS-affected tissues on affected colonies, and unpaired t-tests were used for comparisons between healthy colonies and both types of tissue on affected colonies.

Results

***Siderastrea siderea*.** Healthy corals demonstrated an organized cellular configuration indicative of tissue integrity (Fig. 1A), whereas the DSS-affected tissue exhibited cellular degradation, vacuolization, and necrosis (Fig. 1B). Healthy tissue adjacent to affected tissue on DSS-affected colonies was comparable to tissue on healthy colonies. In healthy calcified tissue, normal aragonite crystals were observed directly adjacent to the calicodermis, and organic matrix was present in decalcified tissue (Fig. 1A). In contrast, aragonite crystals appeared smaller and irregular in calcified tissue from DSS-affected samples, with little organic matrix present in affected decalcified tissue. Numerous zooxanthellae in various stages of cell division were present in the healthy tissue. In DSS-affected corals, there was an apparent decrease in zooxanthellae number, and many appeared abnormal or necrotic, with internal organelle disruption and debris (Fig. 1B).

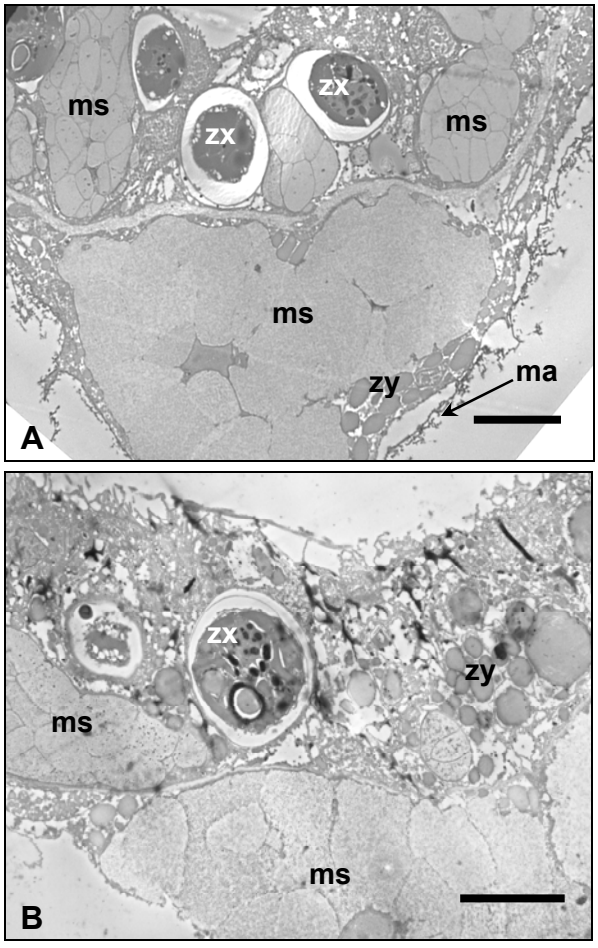

Figure 1. *Siderastrea siderea*, TEM micrographs. A) Healthy gastrodermis; B) DSS-affected gastrodermis. ma: organic matrix; ms: mucocyte; zx: zooxanthellae; zy: zymogen granules. Scale bars: A and B = 10 µm.

In all of the DSS-affected corals, numerous endolithic fungal cells were observed in close proximity to the calicodermis (Figs. 2A & 2B). These fungal cells were associated with swollen, vacuolated mucocytes and an increased concentration of acidophilic/eosinophilic, proteinaceous, electron-dense granules in the calicodermis (Fig. 2A). The morphology and staining properties indicate that these granules are zymogen granules (E. Peters pers. comm., Goldberg 2002). Fungal cells in close proximity to the tissue were not observed in any of the healthy colonies or the samples of healthy tissue from the DSS-affected colonies. Identification and characterization of these fungal cells are ongoing.

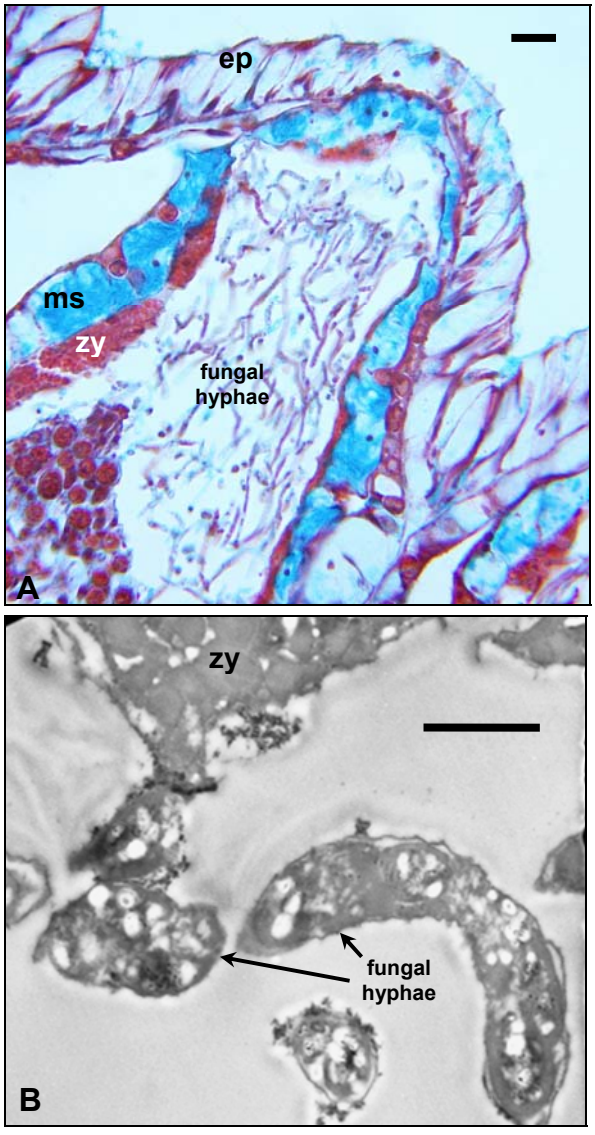

Figure 2. *Siderastrea siderea*. A) Histological micrograph, DSS-affected coral and endolithic fungi; B) TEM micrograph, decalcified DSS-affected coral tissue and fungal cells. ep: epidermis; ms: mucocyte; zy: zymogen granules. Scale bars: A = 20 µm and B = 3 µm

Mucocytes were significantly larger in DSS-affected tissues than in healthy tissues on either healthy (unpaired t=-4.78, df=9, P=0.001) or affected colonies (paired t=3.3, df=3, P=0.046; Table 1). There was no significant difference in the size of mucocytes between healthy tissues on healthy or affected colonies (unpaired t=0.23, df=9, P=0.82).

Table 1. *Siderastrea siderea.* Size of mucocytes (μm^2) in healthy colonies and healthy and DSS-affected tissue on affected colonies.

Tissue type	Mucocyte size (n = cells/colony)
Healthy coral (n=7)	10.3 ± 1.3 (94-236)
DSS-affected (n=4)	26.6 ± 4.1 (62-132)
Healthy tissue on DSS-affected coral (n=4)	9.8 ± 1.5 (150-190)

Agaricia agaricites. Healthy colonies of *A. agaricites* also demonstrated normal cellular configuration (Fig. 3A). The DSS-affected tissue exhibited moderate cellular degradation and vacuolization, particularly of the calicodermis (Fig. 3B). Healthy tissue adjacent to affected tissue on DSS-affected colonies was similar to tissue on healthy colonies. Organic matrix was evident in healthy decalcified tissue. Little organic matrix was present in the DSS-affected decalcified tissue, and aragonite crystals appeared irregular in the affected calcified tissue.

As in *S. siderea*, the most notable difference between affected and healthy tissue in *A. agaricites* was the accumulation of acidophilic electron dense zymogen cells and dispersed granules in the calicodermis and adjacent gastrodermis of DSS-affected tissue (Fig. 3B). Zooxanthellae density appeared to be similar between healthy and affected tissue, and zooxanthellae were observed in various stages of cell division in all of the samples. Zooxanthellae in healthy corals appeared to have a moderately greater intracellular accumulation of starch compared to zooxanthellae in affected corals. This may indicate greater energy demands placed on the zooxanthellae by the coral host in affected corals.

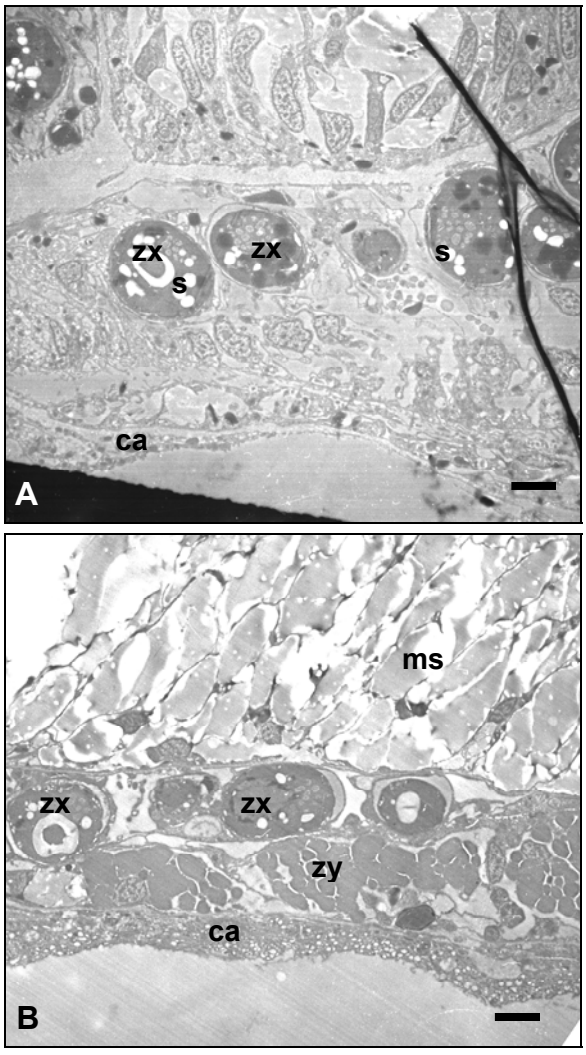

Figure 3. *Agaricia agaricites*. TEM micrographs. A) Decalcified healthy gastrodermis and calicodermis; B) decalcified DSS-affected gastrodermis and calicodermis. ca: calicodermis; ms: mucocyte; s: starch; zx: zooxanthellae; zy: zymogen granules. Scale bars: A and B = 5 µm.

In all of the DSS-affected tissues, numerous protein-staining, unciliated cells were present in close proximity to the calicodermis (Fig. 4). These cells were not observed in any of the healthy corals or in the healthy tissues on DSS-affected colonies. These cells have not yet been identified, although assessment of spatial relationships as well as identification and characterization of these cells is ongoing using fluorescent in-situ hybridization.

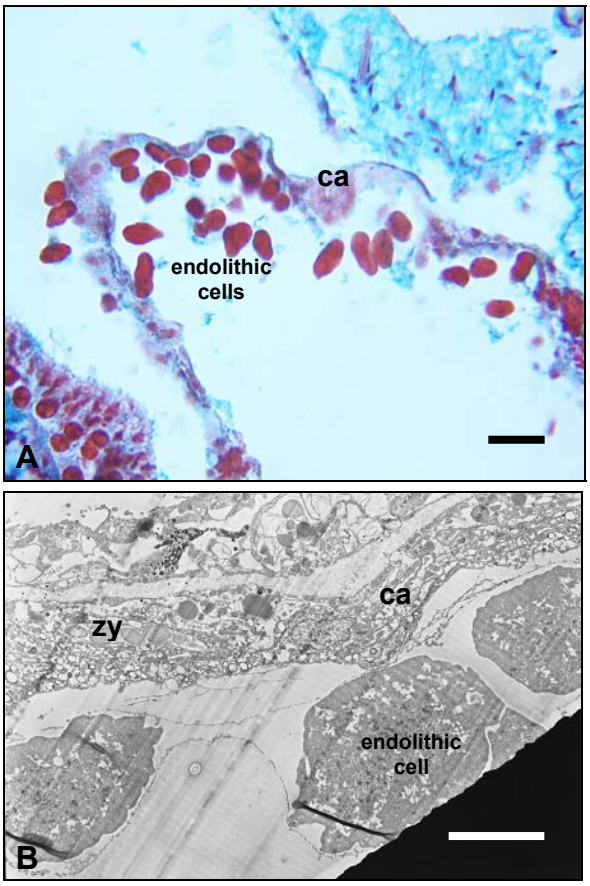

Figure 4. *Agaricia agaricites*. A) Histological micrograph, DSS-affected coral and endolithic cells; B) TEM micrograph, decalcified DSS-affected coral and endolithic cells ca: calicodermis; zy: zymogen granules. Scale bars: A = 20 µm and B = 10 µm.

Discussion

Dark Spot Syndrome causes cellular degradation, vacuolization, and necrosis in affected tissues of *S. siderea* and *A. agaricites*. A concomitant decrease in organic matrix suggests cessation of calcification in affected areas.

An increase in the concentration of proteinaceous, electron-dense inclusions was observed in the calicodermis by histological (Miller et al. 2006) and ultrastructural analysis. The morphology and staining properties indicate that these are zymogen granules. Zymogen granules are normally observed within secretory epithelial cells (granular gland cells), and are reported to function as an aid in feeding. The granules can be released into the gastrovascular cavity for extracellular digestion (Galloway et al. 2007, Goldberg 2002). In the DSS-affected tissues, large zymogen cells were observed in the calicodermis and zymogen granules were often observed free within the calicodermis and adjacent gastrodermis. Galloway et al. (2007) also observed dispersed acidophilic granules associated with endolithic mycosis.

Numerous endolithic fungal cells were observed directly adjacent to the calicodermis in DSS-affected *S. siderea*. Fungal hyphae have recently been observed in association with DSS lesions in several species of Pacific corals (Work et al. 2008) and in *S. siderea* from Florida and Puerto Rico (Galloway et al. 2007). Fungal endoliths are well known from corals and may be pathogenic to their hosts (Le Campion-Alsumard et al. 1995, Bentis et al. 2000, McClanahan et al. 2004). In gorgonians, the fungus *Aspergillus sydowii* causes the disease Aspergillosis, which causes purpling and necrosis of the gorgonian tissue (Smith & Weil 2004). The fungal cells in *S. siderea* are morphologically consistent with the genus *Aspergillus* (Moore et al. 2003); however, their identity has not yet been ascertained.

In contrast, DSS-affected tissues of *A. agaricites* did not contain fungal hyphae. However, a large number of unidentified endolithic cells were observed directly adjacent to the calicodermis. Thus, although DSS-affected tissues in both species exhibit tissue degradation and increased zymogen concentration, the difference in the type of endolithic cells present suggests that the causal agent of DSS in these two coral species may be different. In fact, Weil (2004) suggests that there may be multiple types of DSS (=DSD), and our observations, in concert with differences in zooxanthellar responses in two DSS-affected species (Cervino et al. 2001), suggest that DSS may represent a suite of different conditions.

The increased size of mucocytes along the calicoblastic epithelium in DSS-affected tissues of *S. siderea* suggests the presence of an inflammation response, possibly due to the presence of fungal endoliths. The absence of this response in healthy tissues on affected colonies suggests that inflammation may be localized. The presence of these endolithic cells, coupled with the increased concentration of zymogen granules in adjacent tissues generates questions: is the coral using this digestive enzyme as a defensive mechanism, and does increased concentration of these granules inhibit the endolithic cells or just prevent active invasion of the coral tissue? Although it not yet clear whether the endoliths observed are the causal agents of DSS, our observations suggest that *S. siderea* and *A. agaricites* may utilize a digestive enzyme as a defensive mechanism against endolithic cellular invasion.

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The unknowns in coral disease identification: An experiment to assess consensus of opinion amongst experts

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Abstract: As coral diseases have emerged as a leading cause of mass coral mortality, programmes to research and monitor them have increased. The resultant need to rapidly identify diseases has led to many studies relying on visual analysis and identification of disease. Such approaches have been criticised as subjective and lacking consistency. This study aims to test the level of consistency amongst disease experts by assessing their identification of diseases from a series of photographs. A high degree of variability in almost every photograph was found, with level of observer expertise having no apparent bearing on consistency. It is argued that in order for long term prevalence studies to have meaningful conclusions, standardisation is essential, with recently published coral disease identification guides being a vital step.

Keywords: Coral Disease, Identification

Introduction

Alongside climate change and overfishing, coral diseases are one of the most commonly implicated causes of mass coral mortality (Richardson 1998, Hoegh-Guldberg 1999, Harvell et al. 1999). While partial and whole-colony coral mortality from diseases can act as a natural and sustainable disturbance on coral reefs (Bythell et al. 2000), diseases have in some cases led to long-term, ecosystem-scale impacts. For example, White Band Disease has been implicated in the loss of acroporids as the dominant reef builders in the Caribbean, an unprecedented change within the past 3000-7000 years (Aronson and Precht 2001). Large-scale coral mortality has notable economic impacts for communities reliant on reefs for tourism, fishing and physical protection (Cesar 2002), whilst also contributing to potential phase-shifts and the reduction of reef resilience (Bellwood et al. 2004).

With such serious impacts, it is important that diseases are well studied so that effective management can take place. Yet, despite over 20 years of study, the drivers and dynamics of coral diseases are still poorly understood (CDWG 2007). Whilst it is widely agreed that increased temperature and nutrient enrichment may increase disease prevalence, there is debate as to whether pathogens are acting as primary infectious agents, or are opportunistic, taking advantage of reduced defences of stressed corals (Rosenberg et al. 2007; Lesser et al. 2007). Although over 35 diseases have been described (Lesser et al 2007), etiologies are only understood for five of them, despite considerable recent advances in microbiological techniques (CDWG 2007).

Compounding this lack of understanding is the confusion generated by a lack of rigorous standardisation in disease description and identification. Most monitoring studies do not have the equipment or expertise available for microbial analysis and therefore rely on visual identification (Ainsworth et al. 2007). However, such approaches can be highly subjective and result in errors, a lack of consistency between studies and misidentification (Work and Aeby 2006). Many disease identification guides only provide images of 'classic' well-defined disease symptoms, which means that field workers may not have the diagnostic tools to identify lesions displaying ambiguous symptoms. In these cases, many programmes will still require disease 'identification', perhaps forcing the observer to

categorise disease cases with insufficient or confusing signs. Ainsworth et al. (2007) discovered that what appeared to be a white disease based upon visual morphological characteristics, actually had the microbial characteristics of Black Band Disease, demonstrating that, even aside from subjective errors, visual identification can be unreliable. Regional differences in nomenclature also contribute to variation. For example, swollen pink spots on *Porites* may be called *Porites* trematodiasis in Hawaii, pink spot in Australia, and *Porites* pink blotch disease in Okinawa (Aeby 2006). Highlighting this issue, Richardson (1998) notes that that several coral disease investigators studying an area of reef at the same time named a number of different diseases whilst diagnosing lesions on the same coral colonies.

Recognising the critical nature of accurate field identification, calls have been made to standardise the descriptors of coral syndromes and develop systematic approaches to lesion classification. To this end, Work and Aeby (2006) developed a set of criteria based on the morphological characteristics of a lesion that can be used to describe a syndrome. The most recent system is a handbook and sets of disease identification cards produced by the Coral Disease Working Group (CDWG) that utilise a systematic 'decision tree' approach. This guide is intended to form a standardised guide to identifying, assessing and managing coral reef disease, by providing managers and field surveyors with procedures for describing signs and assessing impacts and giving a standardised approach for recording coral health.

In light of the current debates regarding the best way to monitor coral diseases and the accuracy of visual identification, there is a need to assess the degree of consistency between observers. Therefore, this study aimed to investigate the usefulness of, and provide quantified data on, the level of consistency there is amongst coral disease experts. Using a number of photographs showing a range of coral lesions from different regions, the variation in disease names attributed to each lesion was tested, as well as the extent to which level of expertise affected agreement.

Methodology

The study was conducted through a poster presentation at the 11th International Coral Reef Symposium, Fort Lauderdale, from July 7th to 11th, 2008, as this was where the highest number of disease experts would be present at one time. The poster displayed 23 photographs (Appendix I) showing a variety of coral lesions from the Caribbean and Indo-Pacific regions, but the photograph location was not revealed. One colony photograph of each lesion was displayed and where necessary, an additional close-up showing the polyps was provided. Although it was impossible to select photographs randomly, bias towards 'classic' symptoms, or especially ambiguous samples was avoided and the lesions were chosen for the quality and clarity of the photographs alone. None of the lesions selected had been diagnosed, so there was no 'right' answer to aim for. The images were sourced from the archives of individuals at Newcastle University, UK.

Delegates visiting the poster who had experience of studying diseases were invited to study the photographs and name the disease as best they could. If they were unable to identify the disease, they were advised to leave it blank. The only variable was the level of expertise and respondents were asked to mark one of the five options shown in Table 1. In total, 94 surveys were conducted.

The results were collated according to three different protocols. Firstly, respondent answers were recorded word for word, giving a total of 83 unique descriptions. Secondly, unusual descriptions which had not been heard of were entered into the internet search engine Google Scholar. All descriptions that were not found in publications or monitoring studies were regarded as guesswork and thus re-categorised as 'guess'. Finally, the descriptions that were not a published name, but could reasonably be assumed that the respondent meant a specific disease were grouped into the recognised category. This final data set was analysed in the results.

Table 1: Options for level of coral disease expertise.

1. You have encountered the subject during a short period of academic study or through a brief encounter whilst in the field.
2. You have a couple of weeks experience working on projects in the laboratory or field that have involved disease identification.
3. You have worked on a longer term project(s), encountering a variety of diseases and may have contributed to reports on the topic.
4. Your focus is not coral disease, however, you have spent sustained periods studying coral disease and have produced reports/published on the topic.
5. You have focussed your research in the field of coral disease for a number of years and have published extensively on the topic.

Results

Overall, there was a high variability amongst the respondents when assigning disease descriptions to each photograph, with no photograph receiving less than three descriptions and most receiving over eight (Fig. 1).

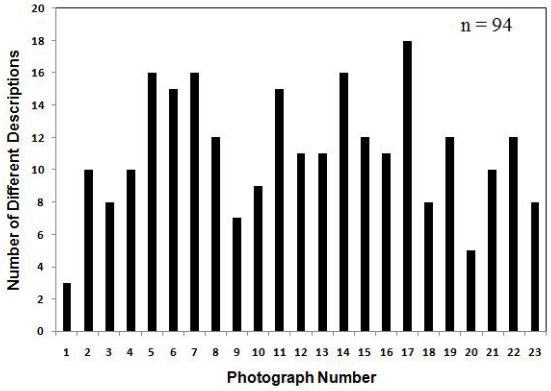

Figure 1: The number of different descriptions assigned to each photograph by whole sample.

This high variability in assigning descriptions to each photograph was also evident within the group of least experienced coral disease experts, with no photograph receiving less than three descriptions and most receiving more than five (Fig. 2). Photograph 17 was assigned eleven different descriptions by respondents, which represents more than one response for every four respondents.

Few signs are shown that consistency of response increases with level of coral disease research experience (Figs. 3 and 4). Despite the sample sizes being lower for the two groupings of respondents with higher levels of coral disease experience there is rarely an increase in response consistency. For instance, the group with most expertise had the highest variance of response for Photograph 5, whilst the group with the least expertise had the lowest variance (Fig. 2). For this photograph those with least expertise (levels 1 and 2) provided approximately one different response for every five respondents, whereas the most experienced (levels 4 and 5) provided a different response for almost every other respondent (Fig. 4). The most experienced respondents also returned a greater number of descriptions than the least experienced group for Photographs 2, 5 and 12, but were most consistent for Photographs 1, 3 and 20.

The spread of disease descriptions for each photograph is shown in Appendix II (Tables 2 and 3).

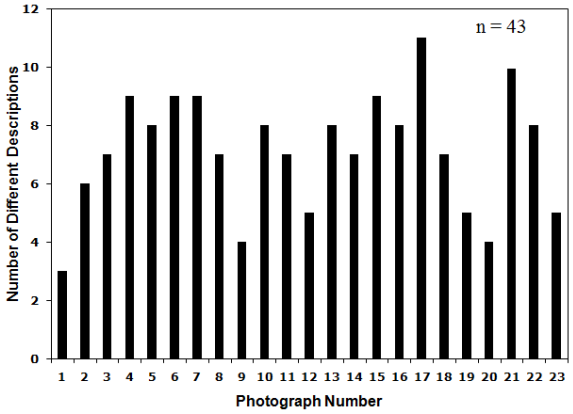

Figure 2: The number of different descriptions assigned to each photograph by respondents - expertise levels 1 and 2.

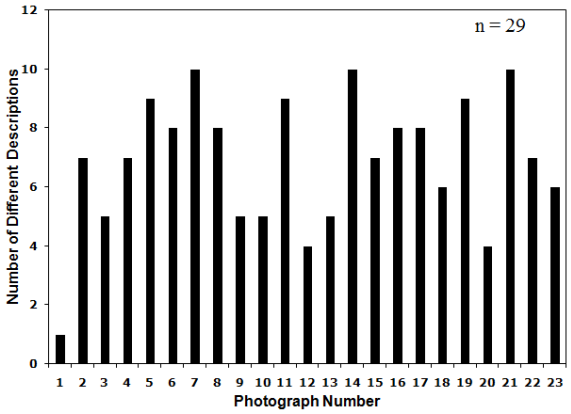

Figure 3: The number of different descriptions assigned to each photograph by respondents - expertise level 3.

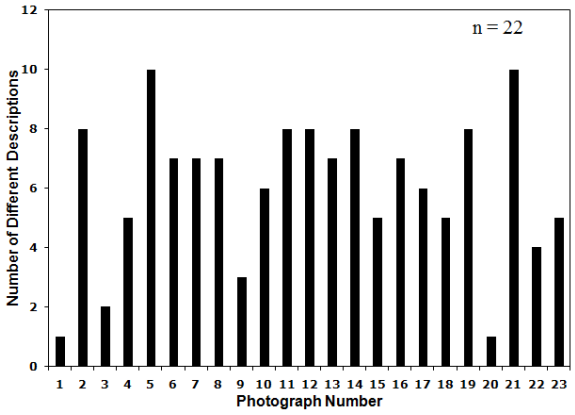

Figure 4: The number of different descriptions assigned to each photograph by respondents - expertise levels 4 and 5.

Some photographs received more consistent overall responses, such as Photograph 1 where 82 of the 94

respondents identified BBD, others identifying brown band and red band (Table 2). Confusion here would appear to be narrowly defined between a range of band diseases, but looking at Photograph 10, it can be seen that confusion can be wider ranging. For this photograph, the most popular responses were bleaching, fish predation and white plague, three syndromes with very different causation (Table 2). Photograph 13 shows that this was also present for the most experienced respondents. In this photograph, experienced respondents were heavily split between a number of white diseases and the very different causation of various predations (Table 3).

Discussion

This study demonstrates that there was a considerable lack of consistency amongst observers when identifying lesions visually. The reason behind such differences is unclear. This could be explained by regional differences in terminology, which are acknowledged to be common (Aeby 2006), and were evident in the initial high number of descriptions. However, this is unlikely to have influenced the final results, as the methods used grouped the similar descriptions in specific categories, which were designed to eliminate such effects as far as possible. The presence of such regional variation raises the question of whether individuals recognise that they are using different names, or whether they regard the different names as different syndromes entirely.

The level of observer expertise did not have a consistent effect on the outcome, so misidentification due to inexperience does not fully explain the results. Although in some cases the more experienced respondents agreed entirely on a specific case description, in others they produced a higher number of descriptions than the least experienced observers. A high degree of variation between observers was therefore likely to be a result of subjective misinterpretation of the information in the photograph, whereby individuals weight syndrome characteristics differently and thus interpret photographs differently.

It is possible that results could have been influenced by how recently an individual was monitoring disease, or by their geographic location. Researchers are more likely to be able to identify diseases in taxa they are familiar with and respondents attempting to identify lesions from unfamiliar regions may explain some of the variability. It is also possible that respondents misrepresented their level of experience, but further study would be required to give clearer answers.

It should be noted that in the field, observers are often looking for a specific set of diseases predefined using histological and microbial tools and will also have more information available than in a two-dimensional photograph, which would probably give *in-situ* observations higher consistency than our results. Also, if using photographs, observers would often have more than one colony photograph providing information, which may improve consistency.

However, it is clear that although the variance of descriptions increases with experience in a few cases, the results strongly suggest that increased training is not the best solution to improve consistency. This highlights the need for standardisation globally, as argued by Work and Aeby (2006). A global standard would go some way to eliminating this variation and reducing the number of descriptions for the same disease.

The results also suggest sizeable problems with attempting to measure disease prevalence changes over time or in larger scale meta-analysis studies. However, progress is being made towards improving this situation and the publication of disease ID guides by the CDWG (2007) offers the beginnings of a solution. If there is widespread adoption of the identification protocols, the likelihood of the misinterpretation and subjective weighting of the importance of lesion characteristics will be reduced and conclusions drawn from monitoring data much more useful for regional and global comparisons. Although such guides will be useful for standardising terminology and field identification, there now need to be studies assessing consistency of identification by observers in the field (i.e. of randomly-selected and representative cases, rather than 'type' cases).

Acknowledgments

The authors are thankful to the following funding bodies: The Joy Welch Educational Charitable Trust, Newcastle University School of Marine Science and Technology, Reef Conservation United Kingdom (RCUK) and the targeted GEF fund of the World Bank.

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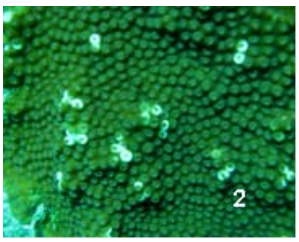
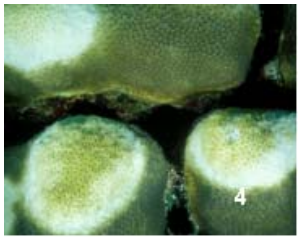
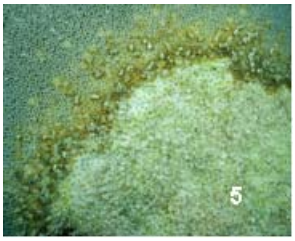
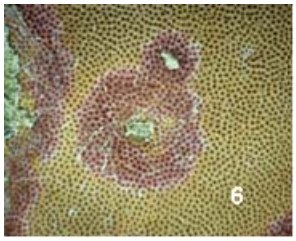
Appendix I

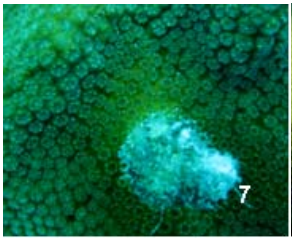
The following 23 photographs are the photographs shown to the respondents in this study. The number assigned to each image is the same as in the original study, thus relate directly to the numbers in all figures and tables in this paper. The images labelled 'b' represent the additional close-ups provided to respondents, where polyps were considered to be hard to identify on the primary photograph.

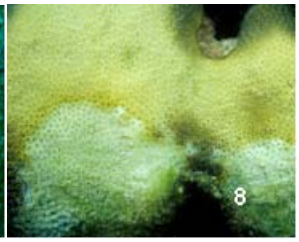


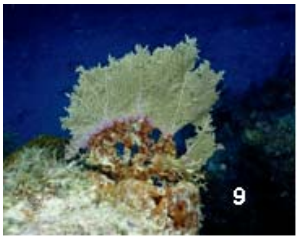


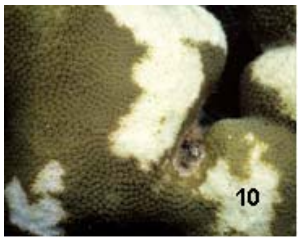








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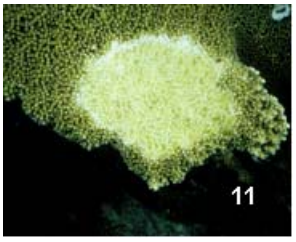
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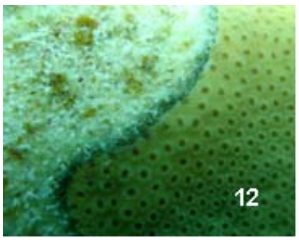
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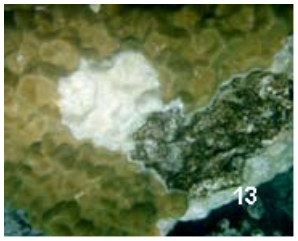
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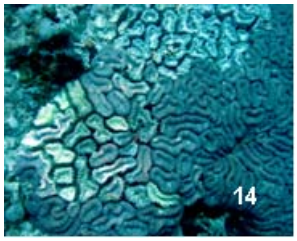
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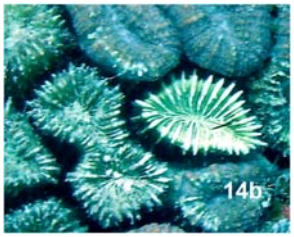
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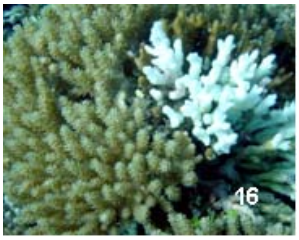
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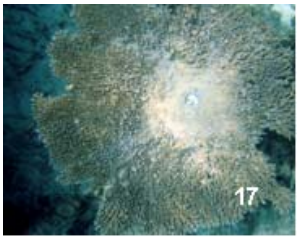
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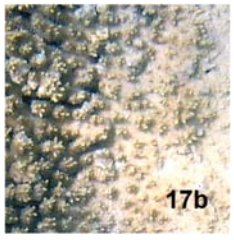
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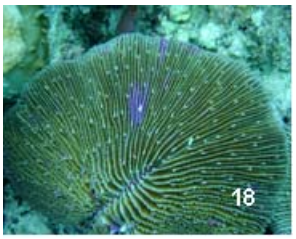
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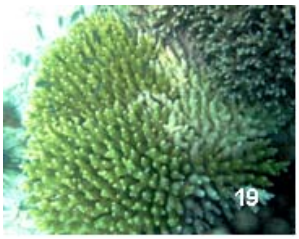
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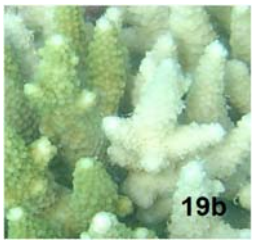
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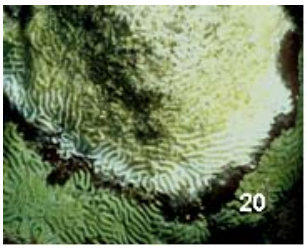
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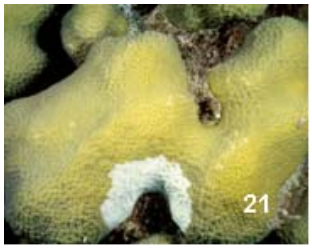
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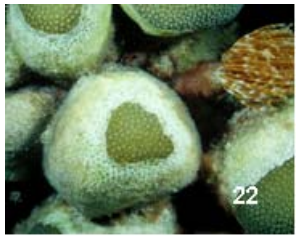
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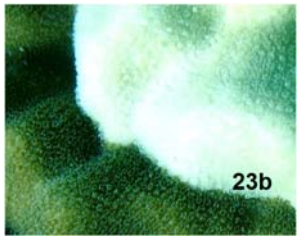
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Appendix II

Table2: Total number of responses per identified description for each photo. Matrix for all respondents.

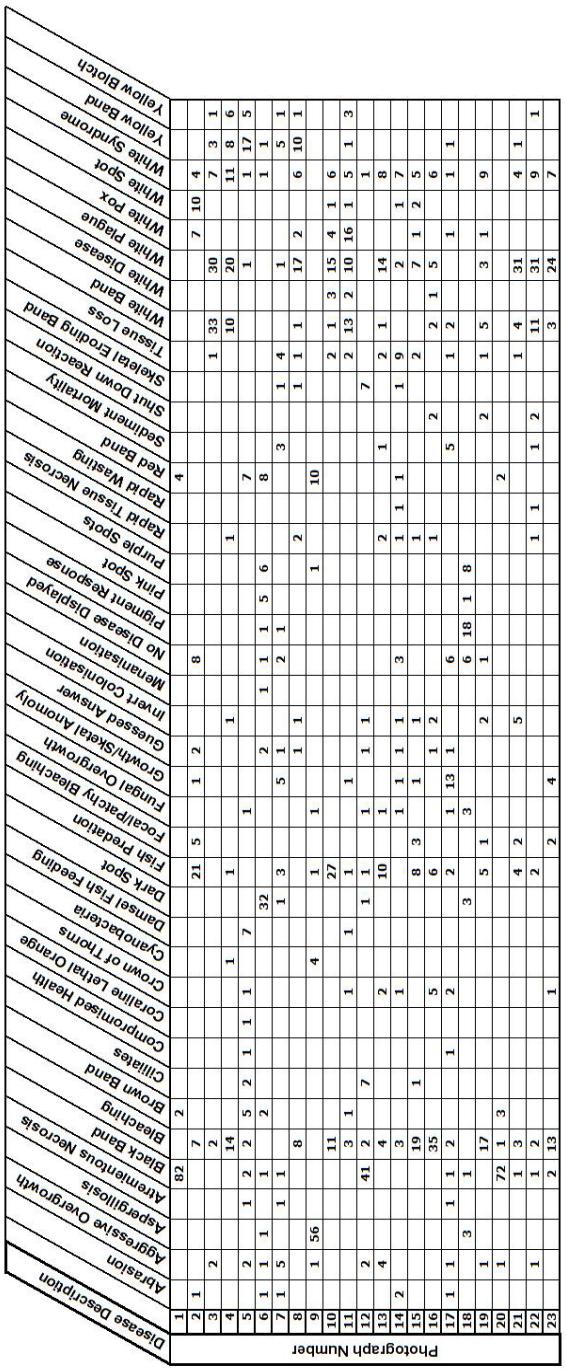
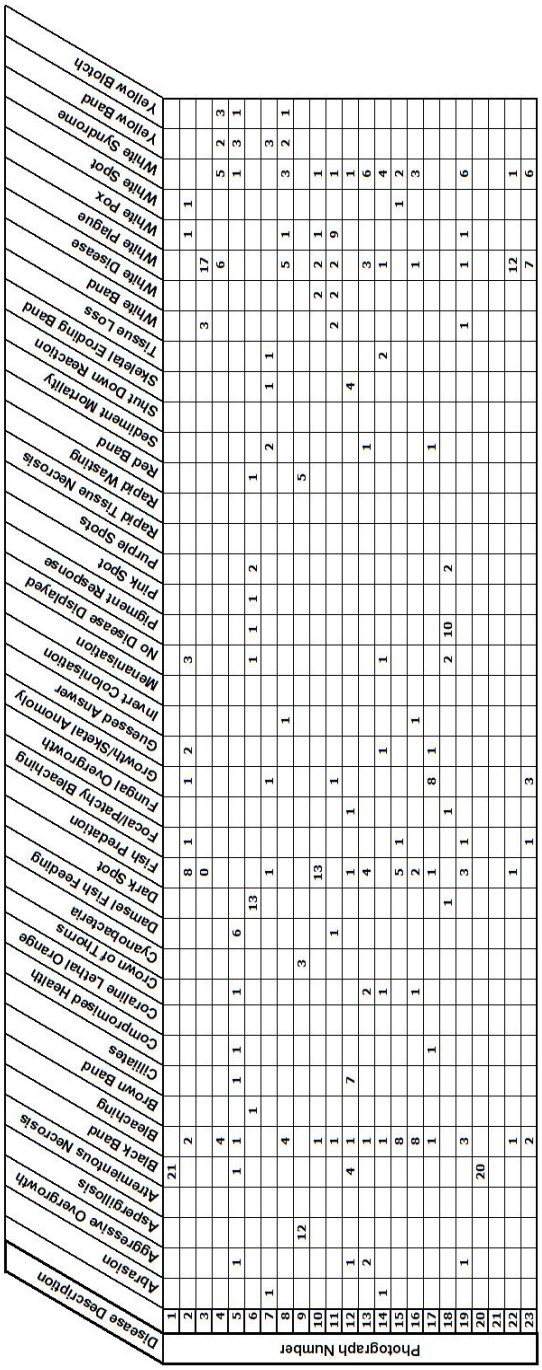


Table 3: Total number of responses per identified description for each photo. Matrix for respondents whom were most experienced in coral disease research (levels 4 and 5).



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Session number 7

Coral and crustose coralline algae disease on the reefs of American Samoa

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Abstract. Surveys for lesions in corals were conducted at seven sites around Tutuila in June 2004 and January 2005. The objectives of the study were to document the distribution and prevalence of disease in the major genera of corals and crustose coralline algae, systematically describe gross and microscopic morphology of lesions in reef corals and determine whether there are seasonal differences in prevalence of disease. We documented 12 different coral disease states from the reefs of Tutuila and two diseases of crustose coralline algae (CCA). *Acropora* white syndrome, *Acropora* growth anomalies and coralline lethal orange disease were the most common diseases on the reefs of Tutuila. No seasonal differences were found in overall prevalence of coral or abundance of CCA disease. Histological analyses of coral lesions revealed that microscopic changes in tissues can be used to distinguish tissue loss due to trauma from changes due to disease, detect micro-organisms associated with certain types of discolorations and found that hyperplasia of the basal body wall was the hallmark microscopic appearance of *Acropora* growth anomalies regardless of gross morphology of tumors.

Key words: American Samoa, baseline disease surveys, coral disease, crustose coralline algae disease

Introduction

Global climate change and human activities are placing coral reef ecosystems at risk. The effects of over fishing and pollution from agriculture and land development have been a major force accelerating decreases in abundance of coral reef species (Pandolfi et al. 2003). Global climate change is compounding these anthropogenic stressors with increased frequency of bleaching episodes and increases in incidence of coral disease predicted (Hoegh-Guldberg 1999, Hoegh-Gulberg et al. 2007). The reefs of American Samoa have been affected by both natural disturbances (crown-of-thorns starfish invasion, hurricanes and mass bleaching events) and human-induced impacts (pollution and over fishing) (Craig et al 2005). The reefs suffered mass bleaching in 1994 (Goreau and Hayes 1994, Birkeland et al. 2000) with reports of bleaching also occurring in both 2002 and 2003 followed by outbreaks of coral disease (Peter Craig, pers. comm.). Earlier qualitative surveys revealed that diseases are present on coral reefs in American Samoa (Work and Rameyer 2005) but no quantitative baseline coral disease surveys had been conducted. A critical component of monitoring the health of reefs is to have baseline 'before' data with which to compare 'after' conditions (Porter et al. 2001, Santavy et al. 2001). We did baseline disease surveys at seven sites around Tutuila in June 2004 and January 2005. These were prompted by anectodal reports of coral disease outbreaks by local conservation agencies in American Samoa. The objectives of the study were to: 1) document the distribution and prevalence of disease in the major genera of corals and distribution and abundance of disease in crustose coralline algae; 2) systematically describe gross and microscopic morphology of lesions in corals 3) determine whether there are seasonal differences in prevalence of disease.

Methods

The distribution and prevalence of diseased corals and crustose coralline algae were documented at seven sites in American Samoa: Vatia, Tafeu, Fagaitua, Faga'alu, Fagatele Bay, Leone and Maloata. To examine potential seasonal differences in disease levels these sites were surveyed in June 2004 and re-surveyed in January 2005. We documented levels of coral and coralline algae disease at each of the sites using two 25 m belt transects with visual counts. The two transect lines were laid end to end along depth contours separated by approximately 5 meters. A team of two divers swam along the transect, with one diver identifying corals to genus and enumerating colonies, while the other diver recorded presence of

disease. Width of the transect was 1-2 m for colony counts and 6 m for disease assessment. Diseased corals and coralline algae were photographed and a general description of the condition recorded (Work and Aeby 2006, Work and Rameyer 2005). Samples of diseased coral (and healthy portions for controls) were collected for histopathological analyses.

Enumeration of individual CCA colonies was not possible so CCA cover was determined using the point-intercept method whereby the substratum underlying the tape measure was recorded at set intervals. At each site one to two stations were surveyed depending on time availability.

For histopathology, corals were processed using standard techniques and stained with hematoxylin and eosin. Special stains were used as appropriate to identify fungi, bacteria, algal filaments, or protozoa. On histology, lesions were classified as depletion of zooxanthella, atrophy, uncomplicated necrosis, necrosis associated with fungi, algae, protozoa, or metazoa, and hyperplasia of basal body wall.

For corals, prevalence of lesions was calculated by extrapolating colony counts within the 25 X 1 m transect to the wider 25 X 6 m disease survey area and using this as the denominator of prevalence calculations, e.g. (number of colonies with lesions/total number of estimated colonies)* 100. Abundance of CCA disease was determined by calculating the number of CCA lesions per square meter of CCA surveyed. Frequency of disease occurrence (FOC) was defined as the number of sites having corals or CCA with disease (within or outside of transects)/ total number of sites surveyed. FOC reflects the spatial distribution of diseases on reefs. Data were not normally distributed even with transformation so a Wilcoxon signed rank test was used to test for seasonal differences in coral disease prevalence and CCA disease abundance. For each survey period (2004 and 2005), a chi square homogeneity test was used to look for differences in the distribution of the number of diseased vs. healthy colonies among the six most common scleractinian genera (*Acropora, Pavona, Porites, Pocillopora, Montipora, Leptastrea*)

Results

Overall occurrence of disease

During surveys in 2004 and 2005, twelve different coral disease states (or lesion types) were documented from eight coral genera on the reefs of Tutuila (Table 1). Coral disease was found at all seven sites each year but the overall proportion of colonies examined that had lesions (prevalence) was low (avg.=0.18%) (range=0.04-0.36%) (Table 1). There were two diseases of crustose coralline algae (CCA) found: coralline lethal orange disease (CLOD) (Littler and Littler 1995) and CCA black fungal disease (Littler and Littler 1998).

Distribution, frequency of occurrence and prevalence of each disease state

Distribution and prevalence of diseases in corals and CCA varied among sites (Table 1). *Acropora* white syndrome (AWS) and *Acropora* growth anomalies (AGA) were the most widespread diseases. AWS was found at 5 of 7 sites (FOC=71.4%) and AGA was found at 4 of 7 sites (FOC=57.1%). The overall prevalence (all sites combined) of each disease was averaged between years and revealed that *Porites diffuse* tissue loss, *Acropora* growth anomalies and *Acropora* tissue loss to be the most commonly encountered lesions (Table 1). Prevalence of *Lobophyllia* diffuse tissue loss, *Pavona* growth anomalies and *Goniastrea* growth anomalies were not calculated as the diseases were present at the study site but not within the belt transects.

CLOD was a common CCA disease found at 4 of the 7 sites (57%) in 2004 and 43% of the sites in 2005 whereas CCA black fungal disease was only found within Fagatele Bay. The number of CLOD infections per m^2 of CCA ranged from 0 to 0.37 and the number of CCA black fungal disease within Fagatele Bay was 0.003 infections per m^2 of CCA.

Difference in disease levels among coral genera

Prevalence of disease differed among the coral genera within each year surveyed (2004: X^2=113.0 df=5, p=0.00; 2005: X^2=29.06, df=5, p=0.00). Of the six most abundant scleractinian coral genera found within our transects, *Acropora* had the highest average prevalence (0.85%) followed by *Pavona* (0.14%), *Porites* (0.11%), *Montipora* (0.06%), *Leptastrea* (0.06%) and *Pocillopora* (0%). One colony with growth anomalies was found in *Goniastrea* and a single colony with diffuse tissue loss in *Lobophyllia* but no signs of coral disease were found in any of the other coral genera that were surveyed.

Seasonal differences in coral and CCA disease on Tutuila

There were no seasonal differences found in coral disease prevalence or CLOD abundance. Average coral disease prevalence was 0.21% (SE$\pm$0.08) in Winter 2004 as compared to 0.15% (SE$\pm$0.05) in Summer 2005 (Wilcoxon signed rank, n=14, p=0.29). CLOD levels were 0.039 (SE$\pm$0.03) CLOD/m^2 CCA in winter as compared to 0.049 (SE$\pm$0.03) in summer (Wilcoxon signed rank, n=14, p=0.88).

Table 1. Prevalence and distribution of lesions in corals and distribution and abundance of lesions in crustose coralline algae. 'X' indicates presence of a lesion at a site outside of the survey area. Data show average prevalence (%) of coral lesions or abundance of lesions for CCA (# lesions/m^2) from surveys in 2004 & 2005 in Tutuila, American Samoa.

	avg. overall prevalence (±SE)	SITES						
		Maloata	Tafeu	Vatia	Leone	Fagatele	Faga'alu	Fagaitua
Acropora growth anomalies	0.47 (0.24)	2.10		1.32	2.90	X		
Acropora ciliate disease	0.04 (0.04)			0.14				
Acropora white syndrome	0.40 (0.24)		1.05	0.81		0.21	0.54	0.27
Lobophyllia diffuse tissue loss	0							X
Porites diffuse tissue loss	0.55 (0.55)					1.00		
Porites multi-focal tissue loss	0.013 (0.013)				0.11			
Pavona endolithic hypermycosis	0.14 (0.14)		0.75					
Pavona growth anomalies	0						X	
Montipora growth anomalies	0.02 (0.02)	0.49						
Montipora endolithic hypermycosis	0.04 (0.04)	0.11	0.12					
Leptastrea growth anomalies	0.06 (0.06)		0.17					
Goniastrea growth anomalies	0				X			
CLOD (# lesions/m^2 CCA)		0.01		0.09	0.02	0.12	0.37	0.04
CCA black fungus (# lesions/m^2 CCA)						0.003		

Histology

We examined tissue specimens from 100 colonies comprising at least 30 species of corals. In some instances, tissue loss in corals was explained by presence of feeding fish (fish bites) or encrusting organisms (barnacles or sponges). Histology in such cases, usually showed abrupt cessation of tissue. In contrast, tissue loss of unexplained etiology (potential disease lesions) usually showed necrosis sometimes associated with algae or sponges. A single plating *Acropora* with a distinct circular pattern of tissue loss revealed microscopic evidence of ciliate invasion into tissues. The gross lesion, as well as the microscopic evidence (ciliates), were both distinct from AWS. Discoloration in corals took two forms (pale and dark purple). The pale discoloration found in *Favia/Favites* was, on microscopy, attributed to mucus sheathing and not considered abnormal. Presence of diffuse, irregular dark purple discoloration in *Pavona* and *Pssamocora* were attributed to overgrowth of skeleton by endolithic fungi leading to necrosis of overlying tissues a disease we term endolithic hypermycosis (Work et al. 2008b). Growth anomalies in both acroporids and montiporids showed proliferation (overgrowth) of the basal body wall. .

Discussion

From surveys in June 2004 and January 2005 we documented 12 different coral disease states from the reefs of Tutuila and two diseases of crustose coralline algae (CCA). *Acropora* white syndrome (AWS), *Acropora* growth anomalies (AGA) and CLOD were the most common diseases on the reefs of Tutuila. White syndrome (defined as focal to multifocal to diffuse, acute to subacute, tissue loss) in *Acropora* is widespread across the reefs of the Indo-Pacific, and has been found from Australia (Willis et al. 2004) out to the remote reefs of the Northwestern Hawaiian Islands (Aeby 2006a, Aeby 2006b). In some regions, white syndrome in acroporids and other coral genera is presumably caused by bacteria within the family Vibrionacae (Sussman et al. 2008). Whether this is the case in other regions remains to be confirmed and further studies are currently underway to examine potential causes of AWS within American Samoa which should shed light on whether the underlying etiology of AWS is similar across regions within the Indo-Pacific.

Acropora growth anomalies (AGA) were common in Tutuila, which is in contrast to the GBR where they are seldom found even though acroporids are the dominant coral (Willis et al. 2004). Work et al. (2008a) compared AGA levels in American Samoa, Johnston Atoll and the northwestern Hawaiian Islands and suggested that occurrence of AGAs may be influenced by environmental factors. Similarly, the occurrence and rate of progression of black band disease are influenced by reduced water quality (Kaczmarsky et al. 2005, Voss et al. 2006).

CLOD, a bacterial disease that kills coralline algae, was originally reported from the Cook Islands in 1993 and subsequently spread throughout the South Pacific including American Samoa where it was first observed in 1995 (Littler and Littler 1995). We found that it is still common on the reefs off Tutuila suggesting it is a chronic disease on these reefs. The black fungal disease found on CCA has a limited distribution in the Indo-Pacific and has only been reported from Tutuila, American Samoa (Littler and Littler 1998).

Prevalence of coral disease was found to be low (avg. <1%) on the reefs of Tutuila, which contrasts with other studies. Dalton et al. (2006)

found disease prevalence ranging from 6.21% to 13.6% on reefs in the Solitary Islands, Australia. Raymundo et al. (2005) surveyed two regions in the Philippines and found the mean disease prevalence to be 8.3%. However, Haapkyla et al. (2007) surveyed reefs in southeast Sulawesi, Indonesia and reported overall prevalence to be 0.57%. Why some regions within the Indo-Pacific seem to be more affected by coral disease than others is still unknown. Coral disease research is in its infancy in the Indo-Pacific and more information is needed on the ecology and causes of coral diseases in order to properly interpret the patterns encountered in the field.

We found *Acropora* to be disproportionately affected by disease in Tutuila. *Acropora* appear to be particularly susceptible to disease and this same pattern is emerging Indo-Pacific wide being found on the GBR (Willis et al. 2004), Sulawesi, Indonesia (Haapkyla et al. 2007) and the northwestern Hawaiian Islands (Aeby 2007). *Acropora* are also highly susceptible to bleaching (Marshall and Baird 2000) and so should be monitored closely in the Indo-Pacific as we face future problems associated with global climate change. Increased sea surface temperatures as well as ocean acidification are predicted with global climate change as well as the resultant increases in coral bleaching and disease (Hoegh-Guldberg 1999, Hoegh-Gulberg et al. 2007).

Seawater temperature can influence disease occurrence. For example, black band disease in the Florida Keys is much more common in the warm summer months and virtually disappears from the reefs during the colder winter months (Kuta and Richardson 1996). In contrast, we found no seasonal differences in overall level of coral or CCA disease for any of the diseases in American Samoa. In Hawaii, the coral disease *Porites* trematodiasis also showed no seasonal variation (Aeby 2007). Differences in seasonal variation among coral diseases may be partially explained by degree of temperature fluxuation within the different regions. For example, the sea surface temperature (SST) in America Samoa only varies between seasons by approximately 2 degrees Celsius whereas in the Florida Keys, SST can vary up to 10 degrees Celsius. Perhaps the small seasonal differences in SST in American Samoa do not affect disease levels as strongly as found in other regions. During the two time periods that we conducted our surveys, the average SST varied by less that one degree Celsius. However, the manner in which a disease responds to a specific environmental stressor will also depend on what is causing the disease (etiology) and the host affected by the disease.

Histology proved to be a critical tool in understanding disease processes and allowed for the the detection of potential causative agents of lesions, particularly in cases where gross lesions had no obvious explanation in the field. For example, tissue loss due to trauma (either through predation or other insult) could be differentiated from tissue loss due to disease based on microscopic changes in tissues such as necrosis and presence or absence of associated factors such as algae, sponges, fungi, or protozoa. We also found that the mottled discoloration observed in faviids was associated with normal mucus sheathing. In some cases, histology also proved useful in understanding the potential etiology of a gross lesion. Dark purple discoloration observed in *Montipora* and *Pavona* was found to be associated with overgrowth of endolithic fungi (Work et al. 2008b). On the other hand, some corals manifesting different types of lesions grossly, had similar microscopic manifestations. For example, six distinct morphological types of growth anomalies were found in *Acropora*, yet on histology, hyperplasia of the basal body wall with absence of or reduced polyp formation was found to be the consistent microscopic change for all types of GA types suggesting perhaps a similar underlying etiology (Work et al. 2008a). In contrast, Domart-Coulon et al. (2006) found normal polyp development in *Porites* growth anomalies emphasizing the utility of histopathological analyses in understanding coral lesions. Comparing in situ photographs with follow-up histological analyses on lesions also provides the opportunity for a more accurate interpretation of lesions in corals in the field.

Future efforts should concentrate on elucidating the mechanisms and potential causes of major diseases on reefs from American Samoa. Given their frequency of occurrence, two candidate diseases for such studies would include growth anomalies and white syndrome in *Acropora* sp.

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Inoculation of *Vibrio spp.* onto *Montastraea faveolata* fragments to determine potential pathogenicity

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Abstract. *Vibrio* strains previously isolated from Caribbean yellow band (CYBD) diseased *Montastraea faveolata* from Puerto Rico were combined into metabolically similar groups based on biolog data (P1, similar to *Vibrio campbellii*; P2, similar to *V. splendidus* and P3, similar to *Aeromonas trota*). Bacterial groups were inoculated onto healthy *M. faveolata* colony fragments that were aerated in individual plastic containers exposed to two temperature regimes (28ºC and 31ºC). Coral genets were divided into two groups of five and maintained at either 28ºC or 31ºC. Three fragments were inoculated with bacterial groups P1, P2, or P3, and two were used as delivery medium or water/container controls at each temperature. Corals were monitored for ten days. After Day 1, two fragments inoculated with P1 and maintained at 31ºC showed signs of yellowing tissue similar to initial stages of CYBD. Three fragments inoculated with P2 and held at 28ºC showed similar signs of paling to yellowing tissue. Two additional fragments showed signs at Day 2 (P1 and P2 at 28ºC). At Day 5, two more P1 inoculated fragments showed disease signs (one at 31ºC and one at 28ºC). Genetic identification of isolates from each group show that members of group P1 are most similar to *V. cambellii* and members of group P2 are most similar to *Photobacterium eurosenbergii*. These preliminary results indicate that more than one type of *Vibrio* sp. may be able to initiate signs of disease and/or bleaching and no clear patterns in response were associated with temperature under the experimental conditions.

Key Words: Yellow band, *Vibrio*, *Montastraea faveolata*, coral disease

Introduction

Among the deleterious effects of climate change on coral reefs has been the increase of coral diseases (Harvell et al. 2004, 2007). Caribbean yellow band disease (CYBD) is particularly prevalent in the Caribbean (Ward et al. 2006; Weil et al. 2006; Weil and Croquer unpub. data), and a similar syndrome occurs in the Indo-Pacific. This disease is temperature influenced (Cervino et al. 2004a), appears to affect zooxanthellae (Cervino et al. 2004b), and is closely associated with species in the genus *Vibrio*. This study tested the pathogenic potential of *Vibrio* isolates obtained from CYBD-infected *Montastraea faveolata* colonies from the south coast of Puerto Rico.

Material and Methods

Bacterial isolates were obtained by SCUBA from CYBD affected *Montastraea faveolata* near La Paguera, Puerto Rico. Samples of the surface mucus and associated coral tissue were collected and *Vibrios* isolated as described in Cunning et al. (this issue). Each isolate was characterized using Biolog™ (Heyward, CA) and identified by sequencing the small subunit 16S rRNA gene as described in Cunning et al (this issue). Isolates were chosen for inoculation based on similarities to isolates previously described (Cervino et al. 2004a) and isolates that were unique to diseased colonies. Isolates fell into one of three groups, corresponding to *Vibrio harveyi/V. campbellii* (P1), *V. splendidus/Photobacterium eurosenbergii* (P2), or *Aeromonas trota/P. eurosenbergiii* (P3) based on carbon source utilization patterns obtained from Biolog™ plates or 16S rDNA sequencing BLAST results, respectively.

Two isolates were used in the P1 group inoculations (3A8 and 1B4), three in P2 (3B7, 1H5 and 3F8) and two in P3 (1A9 and 2H12). Biolog profiles, genetic identities, and GenBank Accession numbers are shown in Table 1. The inocula were made by growing each isolate overnight in GASW broth at 32°C, then combining (1.5ml of each isolate for P1 and P3 and 1.0ml of each isolate for P2) in a test tube with 1g of sterile sieved calcium carbonate sand, shaken, and allowed to absorb on the sand particles overnight (described in Patterson et al. 2002). Ten replicates were made for each inoculum (P1, P2, P3, and controls). Before inoculation, excess medium was decanted and

replaced with sterile seawater. Controls were treated similarly except sterile sand with uninoculated sterile media was used.

Coral fragments were obtained from healthy *M. faveolata* colonies using a hammer and chisel. Fragments were left to acclimate in the field for three days after which each of the 40 fragments were retrieved, tagged, placed in individual aerated containers in a heated water bath either at 28ºC or at 31ºC (Fig.1). Fragments were acclimated in tanks for 24 hours prior to experimental onset. Salinity was checked twice a day and sterile seawater added as needed. Temperature was monitored at least three times a day. An extra five fragments were placed in each temperature, but left untreated. Treatments for each of the coral fragments used in this experiment were randomly assigned. The treated sand grains were poured directly onto the coral fragments. As sand grains were removed from the surface by the corals, inoculate was replaced twice a day using labeled droppers for two days (Fig.2). Observations on yellowing (paling) were made daily (Fig. 3) and the experiment was run for 12 days.

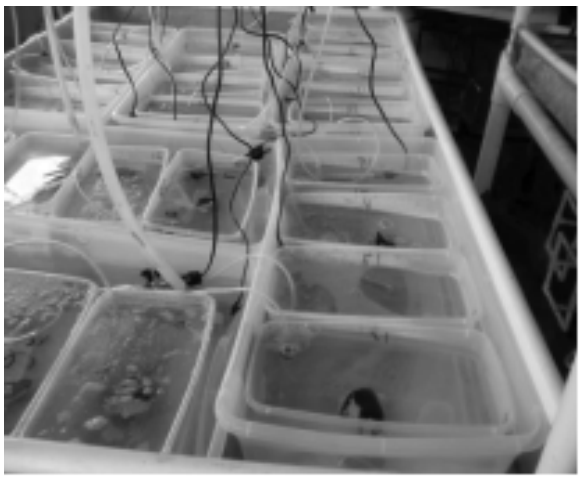

Figure 1: Experimental set up.

Results

Bacteria phylogenetically identical to *Vibrio* spp. previously implicated in YBD (Cervino et al. 2004a) were not identified in the accompyaning study (Cunning et al. this issue). Therefore, *Vibrios* unique to diseased coral that also showed similar carbon source utilization profiles to *Vibrio* species in the Cervino et al. study were subdivided into groups P1-P3 and used in inoculation experiments in this study (Table 1). P1 isolates had slightly different metabolic profiles but were identical to *V. harveyi and V. campbellii*, based on 16S rDNA sequencing results. P2 and P3 isolates were phylogenetically most similar to *Photobacterium eurosenbergii* (Table 1).

Figure 2: Replacement of carbonate inoculate.

After the first day of incubation, two fragments inoculated with group P1 showed yellowing (Fig.3) but only in those fragments incubated at 31ºC.

Conversely, three of the five replicates inoculated with P2 showed yellowing, but only with fragments incubated at 28ºC. This increased to four of the five for P2 (28ºC) on day two and one fragment from P1 (28ºC). On day three, one fragment from the P2 (31ºC) and one from P3 (31ºC) showed yellowing signs. This increased to three of the five for P3 (31ºC) and one for the control (31ºC) on day four. By day 5, all of the P2 (28C) fragments showed signs of yellowing, as well as an additional fragment from P1 (28ºC), P1 (31ºC), P3 (28ºC) and P3 (31ºC). Two additional fragments from the control (31ºC) also showed yellowing by day 5. Throughout the five day period, all fragments from the control (28ºC) remained healthy as did the untreated fragments.

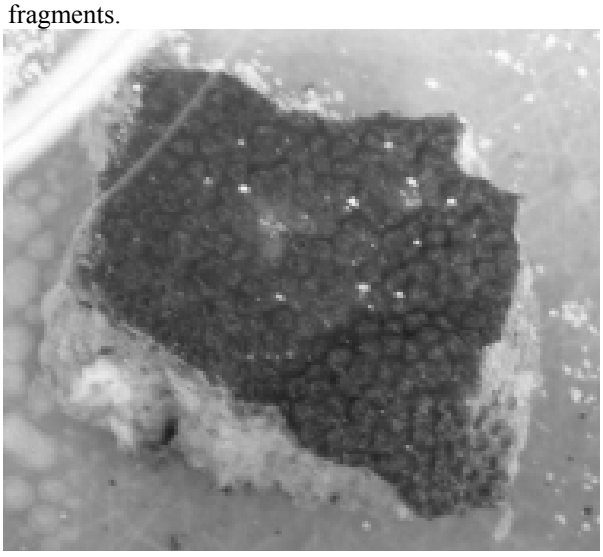

Figure 3: Potential CYBD signs three days after inoculation.

Table 1: Isolates used in Inoculations

Group	Isolate	Biolog™ (Similarity)	% ID based on Genetic Analysis	GenBank Acc#
P1	3A8	*Aeromonas enteropelogens* (0.300) *Vibrio campbellii* (0.130)	100% *Vibrio harveyi/V. campbellii*	FJ356774
P1	1B4	*V. campbellii* (0.230) *Vibrio proteolyticus* (0.280)	100% *Vibrio harveyi/V. campbellii*	EU854881
P2	3B7	*Vibrio splendidus* (0.580) *V. splendidus* (0.770)	100% *Photobacterium eurosenbergii*	FJ356775
P2	1H5	*V. splendidus* (0.500) *V. splendidus* (0.680)	100% *P. eurosenbergii*	EU854888
P2	3F8	*V. splendidus* (0.780) *V. splendidus* (0.410)	100% *P. eurosenbergii*	EU854889
P3	2H12	*Aeromonas trota* DNA group 13 (0.200) *Aeromonas trota* DNA group 13 (0.310)	100% *P. eurosenbergii*	EU854887
P3	1A9	*Aeromonas trota* DNA group 13 (0.250) *Aeromonas trota* DNA group 13 (0.330)	100% *P. eurosenbergii*	EU854886

Although we report here results from the first five days after treatment, the trials were run for twelve days. On day six all fragments in the 31°C table appeared unhealthy (paled) so the heaters were turned off. By day twelve all fragments were unhealthy and the experiment was terminated. Comprehensive results of all inoculations are shown in Figure 4.

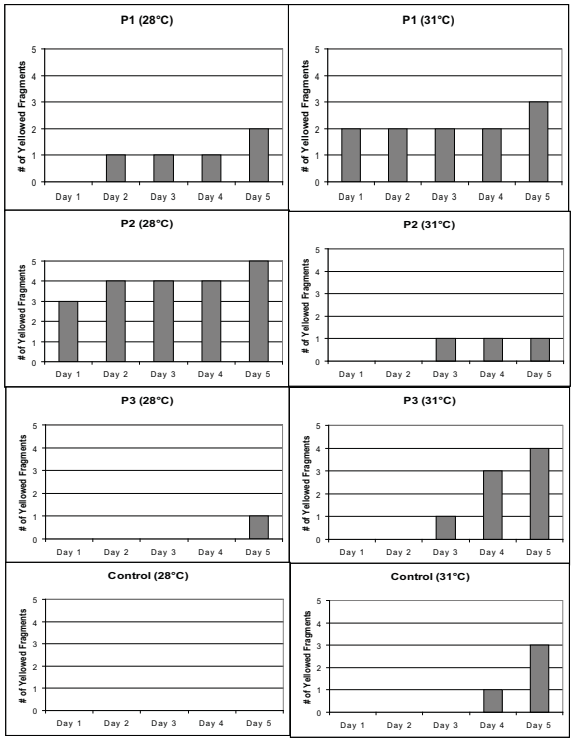

Figure 4: Number of coral fragments showing signs of yellowing over the five day incubation period. Those on the left were kept at 28°C and those on the right at 31°C. P1, P2, and P3 are bacterial inoculants.

Discussion

Of important note is that although isolates chosen for inoculation experiments were identical based on 16S rDNA sequences in the case of Group P1 (*V. harveyi/V. campbellii*) and groups P2 and P3 (*P. eurosenbergii*), however these strains were metabolically different as illustrated by Biolog results (Table 1). This suggests that phylogenetically similar isolates are different strains that could additionally have varying phenotypes with respect to pathogenic mechanisms. Results indicate that, under the experimental conditions, inoculations with one or more of the three bacterial strains in P1 results in yellowing in corals at 31ºC. However some fragments showed yellowing, although to a lesser extent, at 28ºC. A surprising result was that the bacteria in the P2 inoculants greatly hastened yellowing at 28ºC but, conversely, displayed yellowing to a lesser degree at 31ºC. P3 inoculations hastened signs of yellowing by one day at the higher temperature but did not appear significantly different from controls.

Of interest is the potential role of one or more bacteria in the P2 group which hastens yellowing at lower temperatures, but appears to arrest yellowing at higher temperatures. These results suggest that the role of temperature in *Vibrio*-coral interactions may be more complex than previously described.

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A survey of *Vibrios* associated with healthy and Yellow Band diseased *Montastraea faveolata*

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Abstract. Bacteria of the genus *Vibrio* have been implicated in coral bleaching as well as diseases, including Caribbean Yellow Band Disease (CYBD). Four *Vibrio* species have been previously identified as causative agents of CYBD through a series of infection and re-isolation experiments. The mechanisms of pathogenesis and the dynamics of the *Vibrio* community as a whole during CYBD infection are poorly understood. In order to elucidate the role of *Vibrios* in CYBD, a survey of *Vibrio* species associated with healthy corals and CYBD-infected corals was conducted using a culture-based approach. Fragments were collected from CYBD lesions of five *Montastraea faveolata* colonies, five healthy regions of the same diseased colonies, and five entirely healthy colonies. Samples were serially diluted and plated onto TCBS agar to differentially select for *Vibrio* species. Colonies were subcultured using GASWA media and a total of 391 isolates were identified by 16S rDNA sequence analysis. Phylogenetic analysis of *Vibrio* spp. communities of healthy and diseased corals illustrated a shift from isolates taxonomically affiliated with *V. fortis* dominating in healthy corals to isolates taxonomically affiliated with *V. harveyi,* a known marine pathogen, dominating in diseased corals. There was a similar shift to isolates taxonomically similar to *Photobacterium eurosenbergii* as corals progressed to diseased states. However, our study did not find any *Vibrio* species that are always present in CYBD lesions and absent from healthy samples.

Key Words: Yellow Band Disease, *Vibrio*, *Montastraea faveolata*, coral disease

Introduction

Coral reefs worldwide are declining in response to a number of threats, including bleaching and disease (Harvell et al. 1999, 2007). The susceptibility of corals to these threats involves complex interactions among environmental conditions, the coral animal, and its associated algae and bacteria. Some coral-associated bacteria benefit their hosts by providing energy and nutrients and inhibiting pathogens, while others are implicated in coral bleaching and diseases (Rosenberg et al. 2007).

One of the major types of bacteria associated with corals is *Vibrio*. Members of the genus *Vibrio* are highly abundant in the marine environment, and are found in association with a variety of organisms (Thompson et al. 2004). Several *Vibrios* are pathogenic to marine organisms, including *V. harveyi*, which has been implicated in diseases of fish (Ishimaru and Muroga 1997), shrimp (Alvarez et al. 1998), lobsters (Diggles et al. 2000), seahorses (Alcaide et al. 2001), and echinoderms (Becker et al. 2004). *Vibrios* are consistently found in association with apparently healthy corals (Ritchie and Smith 2004; Bourne and Munn 2005; Ritchie 2006), although some have been implicated in coral bacterial bleaching and disease. Ritchie et al. (1994) demonstrated a shift to *Vibrio* dominance in the bacterial communities of bleached corals (>60% *Vibrio*), followed by a return to initial values (~25% *Vibrio*) upon recovery. Apparently healthy non-bleached coral tissue also becomes dominated by *Vibrios* under conditions of increased temperature, suggesting that a shift to *Vibrio* dominance in response to temperature may precede bleaching (Ritchie 2006). Other studies have demonstrated that *V. shiloii* and *V. coralliilyticus* are causative agents of bacterial bleaching in the corals *Oculina patagonica* and *Pocillopora damicornis*, respectively (Kushmaro et al. 1996; Ben-Haim et al. 2003).

In addition to bacterial bleaching, a number of coral diseases have been associated with *Vibrio* species, including white pox, and white syndrome (Rosenberg et al. 2007), gorgonian disease (Hall-Spencer et al. 2007), skeletal tumors in the coral *Porites compressa* (Breitbart et al. 2005), and Caribbean Yellow Band Disease (CYBD) in *Montastraea* spp. (Cervino et al. 2004b).

CYBD has been documented in *Montastraea* spp. around the Caribbean and manifests as pale yellow blotches or bands that spread over the surface of the

coral (Cervino et al. 2004b). Cervino et al. (2004b) identified four *Vibrio* species as causative agents of CYBD through a series of infection and reisolation experiments. The authors suggest the four *Vibrios* act together with *V. alginolyticus* to affect the corals' symbiotic algae and that degradation of zooxanthellae *in situ* results in the pale yellow bands observed on infected Caribbean and Indo-Pacific corals (Cervino et al. 2008). In this study we characterized differences in culturable *Vibrio* communities of healthy and CYBD affected corals.

Materials and Methods

Montastraea faveolata samples were harvested from five entirely healthy colonies (HEA group; HEA-1 to HEA-5), five colonies with CYBD lesions (DIS group; YBD-1-D to 5-D), and five healthy regions of the same diseased colonies (^{H}DIS group; YBD-6-H to 10-H). In addition, one diseased *M franksii* was included in this analysis (YBD-11-D). Sampling was conducted at 10-12m depth at Turrumote reef (17º56.097'N, 67º01.130'W), off La Parguera Natural Reserve on the southwest coast of Puerto Rico in July 2007. Water temperature at the time of sampling was 29.2°C. Samples were shipped overnight to Mote Marine Laboratory, Sarasota, FL, where mucus samples were removed, serially diluted and plated onto thiosulfate-citrate-bile salt (TCBS) agar to differentially select *Vibrios*. Vibrio colonies were then subcultured using glycerol artificial seawater agar (GASWA; Smith & Hayasaka 1982) and 391 isolates were selected for genetic analysis. DNA was extracted by boil lysing in 100 µL of sterile water at 95°C for 10 minutes. PCR amplification of the 16S rRNA gene was carried out in 50 µL reactions using 1x PCR buffer (Qiagen), 200 µM of each deoxynucleotide (Qiagen), 2 µM each of 16S rRNA primers U9F (5'-GAGTTTGATYMTGGCTC-3') and U1509R (5'-GYTACCTTGTTACGACTT-3') (Invitrogen), 1U Taq polymerase (Qiagen), and 2 µL DNA template. Thermocycling began with an initial denaturation step at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30s, annealing at 50°C for 30s, and extension at 72°C for 90s, with a final extension step at 72°C for 10 min. Amplification of a ~1500 bp fragment was confirmed by electrophoresis on a 1% agarose gel. PCR products were sequenced by Macrogen, Inc (Korea). Consensus sequences from forward and reverse strands were generated and GenBank BLAST searches were performed to demonstrate percentage identity to known bacteria. Sequences were aligned using CLUSTAL X version 1.83 and the ends were trimmed resulting in ~1325 bp sequences for phylogenetic analysis. A pairwise comparison was used to eliminate duplicate sequences. In accordance with current *Vibrio* taxonomical standards (Kita-Tsukamoto et al. 1993, Thompson et al. 2005) sequences with >99.3% similarity were considered representatives of the same taxon and sequences were grouped by this criterion for phylogenetic analysis. Neighbor-joining dendrograms were constructed using MEGA software (v.4) with bootstrap values based on 1000 replicates. GenBank Accession numbers are as follows: DIS (EU854819-EU854889, and FJ356774-5), ^{H}DIS (EU517610-EU517657) and HEA (EU854890-EU854953).

Results

A total of 391 16S rRNA gene sequences were obtained from bacterial isolates, 215 from DIS samples, 108 from ^{H}DIS samples, and 68 from HEA samples. Successful sequences were derived from a very small proportion of 2 of the 5 HEA samples (Fig. 2). For this reason, these data were omitted to avoid statistical error.All sequences were taxonomically similar to five *Vibrio* taxa: *V. harveyi*, *V. fortis*, *V. brasiliensis*, *V. chagasii*, and *Photobacterium eurosenbergii* (Fig. 1). Isolates similar to *V. brasiliensis* and *V. chagasii* constituted 0.47% and 3.74% of DIS isolates, respectively, but were not present in ^{H}DIS or HEA samples. Isolates taxonomically similar to *P. eurosenbergii* made up 3% and 4% of DIS and ^{H}DIS samples, respectively, but were not present in HEA samples. *V. fortis*- and *V. harveyi*-like isolates were present in all three groups, with those similar to *V. fortis* constituting 69% of HEA isolates, 58% of ^{H}DIS isolates, and 47% of DIS isolates, and those taxonomically similar to *V. harveyi* constituting 31% of HEA isolates, 38% of ^{H}DIS isolates, and 45% of DIS isolates (Fig.1).

Due to considerable variation in the relative proportions of *Vibrio* taxa among individual samples within each group (Fig. 2) there were no significant differences in mean proportions of taxa among groups. Among DIS samples, the proportion of *V. harveyi*-like isolates ranged from 0.161 to 0.788 (mean=0.447±0.096), and that of *V. fortis*-like isolates ranged from 0.212 to 0.742 (mean=0.491±0.095). *V. chagasii*-like isolates were present only in sample YBD-1-D, and *V. brasiliensis*-like isolates were only present in sample YBD-3-D. *P. eurosenbergii*-like isolates were present in every DIS sample except YBD-3-D and YBD-11-D. Among ^{H}DIS samples, the proportion of *V. harveyi*-like isolates ranged from 0.154 to 0.522 (mean=0.330±0.071), and that of *V. fortis*-like isolates ranged from 0.435 to 0.818 (mean=0.632±0.071). *P. eurosenbergii*-like isolates were present in all ^{H}DIS samples except YBD-7-H. Only the HEA samples showed any consistency, with *V. fortis* dominating and *V. harveyi* being the only other isolate present in all three samples. A fourth healthy colony was sampled but not included in analysis because from it only five sequences were ob-

tained. However, it is worth noting that a sequence similar to *Vibrio coralliilyticus*, a known coral pathogen, was found in this sample.

Discussion

In this study we have addressed culturable members of the genus *Vibrio* for analysis. Although it is estimated that less than 1% of bacteria can be cultured in the laboratory, culture based work is the only way to address pathogenesis in coral diseases. Drawbacks to this work include an inability to culture various *Vibrios*, which could enter a viable but non-culturable state during infections rendering them non-detectable using these methods. We would stress that this study does not provide quantitative data of *Vibrios* present, but only an estimate of proportional abundance. When sequences are pooled into DIS, ^{H}DIS and HEA groups (Fig. 1), several differences are apparent. First, the proportion of *V. harveyi*-like sequences is lowest in the HEA group, higher in ^{H}DIS, and highest in DIS, indicating that *V. harveyi*-like isolates become more abundant as the disease progresses. *V. harveyi* has been shown to be a pathogen to many marine organisms (Austin and Zhang 2006) and produces a variety of toxins including haemolysins (Zhang et al. 2001), which can lyse cells and cause tissue damage (Zhang and Austin 2005). Involvement of these haemolysins in CYBD etiology would account for tissue lesions observed on infected corals and would support indications that lysis of zooxanthellae may be a potential disease mechanism (Cervino et al. 2004a, b). However, *V. harveyi* is also found in healthy corals (present study and Ritchie 2006), thus any involvement in CYBD must be opportunistic or mediated by a mechanism such as quorum sensing (Thompson et al. 2004).

In addition to an increase in *V. harveyi*-like isolates as corals progress to diseased states, there is a concurrent decrease in *V. fortis*-like isolates, suggesting *V. fortis* may be a dominant and innocuous member of the healthy *Vibrio* community that is outcompeted as the disease progresses. However, *V. fortis* has also been shown to be a pathogen to both fish and crustaceans (Austin et al. 2005). Additionally, an isolate with >99.5% 16S sequence similarity to *V. fortis* has exhibited algicidal activity (Imai et al. 2006), which is

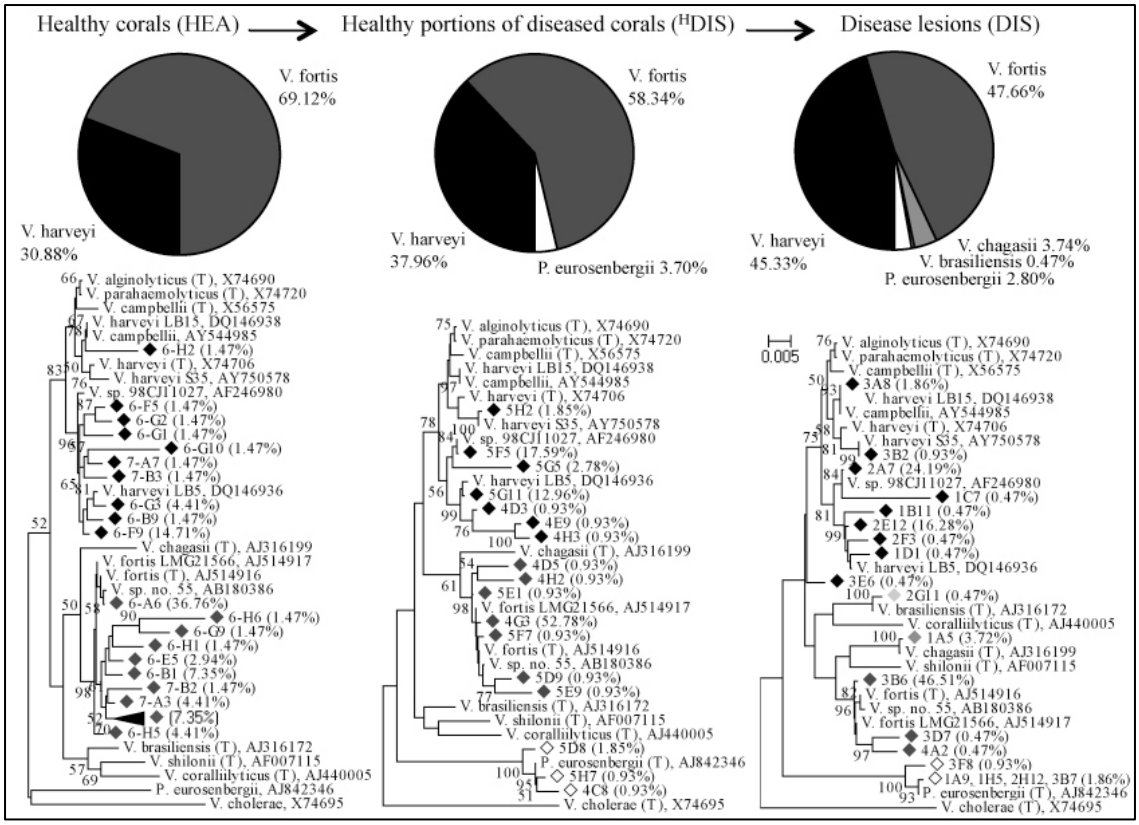

Figure 1. Phylogenetic trees and pie charts representing phylogenetic positions and relative abundances of major groups of *Vibrio* isolates identified from HEA, ^{H}DIS, and DIS groups by 16S rDNA sequence analysis of cultured isolates. Branches marked by diamonds represent sequences from the present study (Genbank accession numbers EU854819-EU854953). Percentages indicate the proportion of sequences in that group that are >99.3% similar. Other sequences included are the closest matches from GenBank, (T) indicating type strain. Dendrograms were created in MEGA (version 4) using the Neighbor-Joining method. Numbers at the nodes indicate the level of bootstrap support (%) from 1000 replicates. The evolutionary distances were computed using the Maximum Composite Likelihood method and the scale represents 0.005 substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). Pie charts represent the proportion of all sequences from HEA, ^{H}DIS, or DIS groups that match most closely to *V. harveyi, V. fortis, V. brasiliensis, V. chagasii,* or *P. eurosenbergii*.

a proposed mechanism of CYBD infection (Cervino et al. 2004a, b). However, as *V. fortis* was dominant in healthy corals in this study, its involvement in CYBD must also be opportunistic.

In addition to having more *V. harveyi*- and less *V. fortis*-like sequences, the DIS group contained sequences similar to *V. brasiliensis* and *V. chagasii* that were not present in ^{H}DIS or HEA samples. *V. brasiliensis,* isolated first from bivalve larvae (Thompson et al. 2003) is closely related to the oyster pathogen *V. tubiashii* and the coral pathogen *V. coralliilyticus* (Thompson et al. 2004) and has been shown to be pathogenic to fish and crustaceans (Austin et al. 2005). *V. chagasii* is a chitonlytic bacterium that has been isolated from the gut of turbot larvae (Thompson et al. 2003) and coastal fishes (Itoi et al. 2006). The presence of these sequences only in DIS samples is consistent with increased bacterial diversity observed in other coral diseases (Pantos et al. 2003).

Another difference among the three groups was the presence of *Photobacterium eurosenbergii,* which has been associated with bleached corals on the Great Barrier Reef (Thompson et al. 2005), in DIS and ^{H}DIS groups, but not in the HEA group.

Although the pooled analysis shows differences among the three groups, there is inconsistency among individual samples within groups (Fig. 2). For example, in the DIS group, three samples were dominated by *V. harveyi*, and three by *V. fortis*. *V. brasiliensis* and *V. chagasii* were each only present in one out of six DIS samples. ^{H}DIS samples showed slightly more consistency, with four dominated *V. fortis*, and one by *V. harveyi*. *P. eurosenbergii* was present in all but one of the DIS and ^{H}DIS samples. The HEA group showed more consistency, each with *V. fortis* dominating. This suggests healthy corals have a more stable *Vibrio* community that subsequently becomes unstable and variable during CYBD infection.

Despite variability in their constituents and proportions, the *Vibrio* communities in healthy and diseased portions of the same coral colonies show a consistent difference from one another. Colonies in which disease lesions were dominated by *V. harveyi* had a higher proportion of *V. fortis* in the healthy portions. Conversely, colonies with more *V. fortis* in the CYBD lesions had a higher proportion of *V. harveyi* in the healthy portions. Thus, the only common difference between healthy and diseased portions within a colony is that relative proportions of *Vibrio* taxa have changed. Thus, a disruption of the *Vibrio* community is always associated with the disease, but the magnitude and direction of this disruption is not consistent.

Previous studies on CYBD have suggested a specific group of *Vibrio* isolates not found in healthy corals are the causative agents of CYBD (Cervino et al. 2004a, b). However, our study did not find any *Vibrios* that are always present in CYBD lesions and always absent from healthy samples. Instead, we show that there is considerable variation in the community of *Vibrios* associated with the disease. A study of bacterial communities associated with white plague has shown similar variability, and has suggested there may be several different potential pathogens (Pantos et. al 2003). Our results suggest a variety of bacteria may be able to induce disease symptoms, and that the mechanism of disease may be a toxin that is widespread among *Vibrios*, such as haemolysins (Wang et al. 2007).

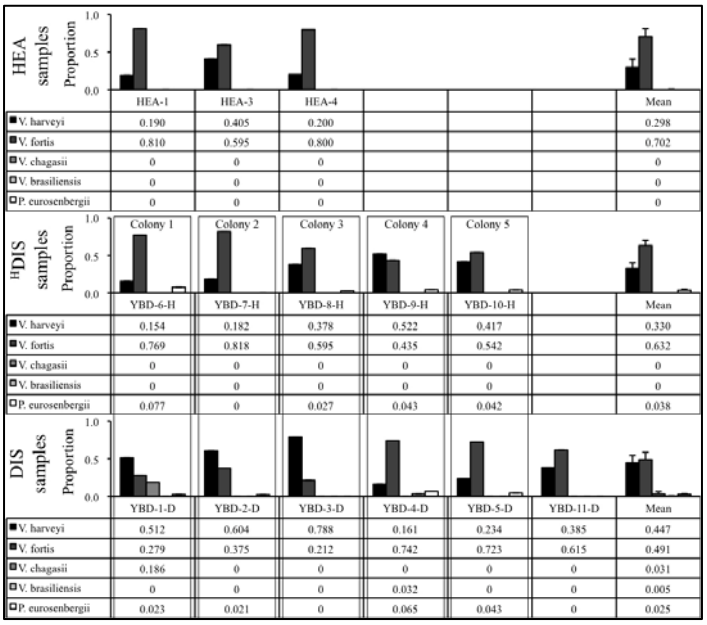

Figure 2. Relative proportions of major *Vibrio* taxa in each sample within groups (HEA (n=3), ^{H}DIS (n=5), and DIS (n=6)). Means represent the average proportion of each *Vibrio* taxon in all samples within each group, and error bars represent standard error of the mean. Rectangles surround samples from ^{H}DIS and DIS groups that originated from the same coral colony. Note that only 3 entirely healthy colonies resulted in the successful 16S sequencing of cultured *Vibrios*. Results in this figure include the individual *M. franksii* YBD sample,

The variation in the *Vibrio* community associated with CYBD may also suggest that *Vibrio* taxa are working together as pathogens, and may do so regardless of their relative proportions. Supporting this is the fact that relatedness among bacteria leads to an increase in virulence (Foster 2005). Also lending support to this theory is the fact that in the CYBD infection experiments conducted by Cervino et al. (2004b), disease symptoms were only induced when multiple *Vibrio* strains were inoculated as a group. Understanding the dynamics that may cause disease symptoms, especially by groups of opportunistic microbes, will be an important new area of research. The identification of opportunistic pathogens requires establish-

ing both pathogenicity and the conditions which facilitate opportunistic colonization. Future studies should include quantitative studies of bacterial abundance by methods such as Real Time PCR technology, pathogenicity exposure experiments and development of novel tools to diagnose pathogenic strains of CYBD.

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Development of a Novel Method for Coral RNA Isolation and the Expression of a Programmed Cell Death Gene in White Plague-Diseased *Diploria strigosa* (Dana, 1846)

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Abstract. Coral diseases are significantly contributing to the degradation of tropical coral reefs throughout the world. A majority of research on coral diseases conducted to date has focused on microbiological and ecological surveys to characterize coral associated microbiota and factors contributing to disease prevalence. Recent studies have shown that corals use innate immunity to resist microbial invasions, but the mechanisms controlling these defenses remain poorly understood. In this study we investigated molecular responses to disease manifested by corals during periods of infection. During July of 2007 in Cayos Cochinos, Honduras, surface layer mucus samples were collected from colonies of diseased and healthy *Diploria strigosa,* putatively affected by White Plague Disease. Coral RNA was isolated from condensed mucus samples stabilized in RNA*later* (Ambion), and differential gene expression was analyzed by differential display PCR. PCR products were analyzed qualitatively by 2 % agarose gel electrophoresis, and candidate immunity genes were isolated, cloned, and sequenced. Derived sequences had similarities to immunity genes of other invertebrates, including programmed cell death. The results of this study contribute to the characterization of molecular mechanisms controlling coral immune responses to disease infection, and present the use of a novel and non-invasive method to collect coral RNA.

Keywords: Coral Disease, Immunity, Gene Expression, Differential Display PCR, Programmed Cell Death

Introduction

Since the first report of coral disease over three decades ago, research has been largely dominated by ecological and microbiological investigations (Pantos et al. 2003, Sekar et al. 2006, Santavy et al. 2001). Although these studies are necessary to characterize coral-associated microbiota and ecological factors influencing disease prevalence, it remains unclear how coral innate immune defenses contribute to disease resistance and susceptibility.

Studies of coral innate immunity are just beginning to reveal the repertoire of defenses against microbial invasions. In gorgonians, inflammatory responses involving the activation of amoebocyte differentiation and proliferation, melanin deposition, chitinases and antioxidants have been observed (Mydlarz et al. 2008, Douglas et al. 2007, Mydlarz et al. 2006). Programmed cell death pathways have also recently been identified to have a putative role in White Syndromes observed on the Great Barrier Reef (Ainsworth et al. 2007). Immunity genes that are conserved among different phyla have been identified in corals (Miller et al. 2007), but a characterization of their functions in coral innate immunity has not been reported.

The coral surface mucus layer (SML) is an important component of the coral innate immune system. It is a physical barrier that is the first line of defense against potential pathogens in the water column (Sutherland et al. 2004). Periodic sloughing of the SML and the production of antimicrobial compounds are important mechanisms for regulating coral-associated microbial communities (Brown and Bythell 2005). Differential antimicrobial properties and microbial communities of the SML of diseased and healthy corals have been observed (Gil-Agudelo et al. 2006, Ritchie 2006, Pantos et al. 2003).

In this paper, we present an investigation of differential gene expression between colonies of *Diploria strigosa* which are healthy and which are putatively infected with White Plague using coral cells sampled from SML.

Materials and Methods

Field sample collection

A survey of coral disease prevalence was conducted on a shallow patch reef area of the Plantation Beach

Reef of Cayos Cochinos, Honduras (Fig. 1). Three *Diploria strigosa* colonies putatively affected by White Plague and three apparently healthy colonies were selected for sampling between 0400 and 0500 hours before civil dawn (Table 1). Samples from the SML of diseased colonies were collected from areas adjacent to the disease lesion and apparently healthy tissue at least 20 cm from disease lesion. Mucus samples were collected from central areas of healthy colonies unaffected by disease.

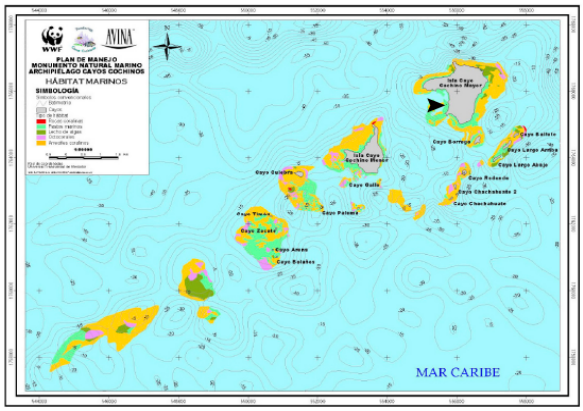

Figure 1: Map of Cayos Cochinos, Honduras. The black arrow indicates the Plantation Beach Reef area, where coral mucus samples were collected for this study (HCRF/WWF 2004).

Excess mucus production was induced at the sampling sites of the coral colonies by rapidly ejecting seawater from sterile 10 cc syringes with no needles 2 to 3 cm from coral surface. Duplicate 10 ml mucus samples were collected with sterile syringes from each sampling area. Samples were kept at ambient seawater temperature (~27°C) for no more than an hour, stored on ice, and processed upon return to shore.

Mucus samples were condensed using a hand crank centrifuge (~300 rpm) for approximately 5 min, and the resulting pellets were removed to 1.5 ml microcentrifuge tubes and stabilized in 10x volumes of RNA*later* (Ambion). Stabilized samples were stored in a conventional freezer for up to three weeks, transported at room temperature for 24 hours, and stored at -80°C until molecular analyses were performed at the laboratory in Sarasota, Florida.

RNA isolation and cDNA synthesis

Stabilized mucus samples were centrifuged (10,000*g*) for 20 min at 4°C, the RNA*later* supernatant was removed, and the resulting mucus pellets were individually homogenized in Trizol (Invitrogen). Contaminating polysaccharides, proteins and genomic DNA were removed by centrifugation (10,000*g*) for 10 min at 4°C. Total RNA was extracted from the Trizol-mucus homogenate supernatant, according to the manufacturer's protocols. One µl glycogen (1 mg l^{-1}) was added to enhance the precipitation of low concentrations of RNA with 99.5% isopropyl alcohol.

Sample #	Colony #	Condition	Area of Sample Collection
7	2	WP	Disease Front
9	2	WP	Healthy Tissue
13	9	WP	Disease Front
15	9	WP	Healthy Tissue
18	4	WP	Disease Front
22	4	WP	Healthy Tissue
23	A	ND	Healthy Tissue
25	B	ND	Healthy Tissue
28	C	ND	Healthy Tissue

Table 1: Description of mucus samples collected from *D. strigosa* and analyzed in this study (WP = white plague-like disease, ND = not diseased). Matching numbers under the colony column indicate samples collected from the same *D. strigosa* colony.

For each total RNA sample, four separate SuperScript III reverse transcriptase reactions (Invitrogen) were performed with one of the following primers: anchored-dT primers with HindIII restriction sites (5'AAGCTTTTTTTTTTT-N-3'; N = A, G, or C) and a mixture of random hexamer primers (Invotrogen). A negative control reaction with random hexamer primers and total RNA was conducted without the addition of reverse transcriptase, and the products of this reaction were used in coral gene-specific RT-PCR to ensure that there was a negligible level of genomic DNA contamination in the RNA samples (methods described below).

Coral gene-specific RT-PCR

Random hexamer primed cDNA was used as a template for coral gene-specific reverse transcriptase PCR (RT-PCR). PCR primers specific to cytochrome-B (Cyt-B) and β-tubulin (Tub) genes were designed from sequences available for *Manicina areolata* and *Diploria labyrinthiformis* (Accession # AB117305.1 and AB118249.1, respectively), which have sequences highly conserved with *D. strigosa*. RT-PCR was conducted to confirm the presence of coral RNA sequences in the mucus samples, which were likely to be dominated by microbial sequences. Tub and Cyt-B amplicons were sequenced to ensure these genes can be used effectively to confirm the presence

of good quality coral gene sequences in the mucus samples.

Differential display PCR
Anchored-dT primed cDNA was used as a template for differential display PCR (DD-PCR), modified from the methods first presented by Liang and Pardee (1992). Five combinations of arbitrary 13-base primers with Hind-III restriction sequences and the anchored-dT primer used to synthesize the cDNA template were used to amplify a subset of the transciptome present in each cDNA sample. DD-PCR products from samples originating from disease lesions, healthy areas of diseased colonies and healthy colony controls were separated side-by-side by 2% agarose gel electrophoresis for each primer combination. Gels were stained with ethidium bromide and DNA bands that were differentially amplified were excised for cloning and sequencing.

Sequencing candidate molecular responses to disease
Excised bands were extracted from the agarose gel using Wizard SV Gel and PCR Clean-Up System according to the manufacturer's protocols (Promega). The extracted DNA was cloned into a TOPO TA plasmid and transformed into *Escherichia coli* (strain DH5α-T1^{R}) with an Invitrogen kit for sequencing, according to the manufacturer's protocols. Plasmids were extracted from cultures of individual transformed *E. coli* colonies using Quick Plasmid Miniprep kit (Invitrogen), and the cloned DD-PCR inserts were sequenced using a standard automated Sanger sequencing protocol. Similarities of the derived sequences in this study to sequences available in the National Center for Biotechnology Information were found using a basic local alignment search tool (BLAST).

Results

Coral gene-specific RT-PCR
Unlike the results reported by Santiago-Vazquez and colleagues (2006), we were able to store coral nucleic acids successfully using RNAlater. Eight of nine samples analyzed in this study provided consistent positive results for the presence of coral nucleic acids, confirmed by the amplification of both Cytochrome-B and Tubulin genes (Table 2 and Figure 1). Amplicons were not observed for negative control reactions conducted with total RNA templates exposed to reverse transcriptase reaction conditions.

Sample #	Cyt-B	Tub
7	+	+
9	+	+
13	-	+
15	+	+
18	+	+
22	+	+
23	+	+
25	+	+
28	+	+

Table 2: Summary of results from coral gene-specific RT-PCR. Respectively, (+) and (-) indicate successful and unsuccessful amplification of coral Cytochrome-B (Cyt-B) and β-Tubulin (Tub) genes. Sample # refers to the original mucus sample from which the cDNA template originated.

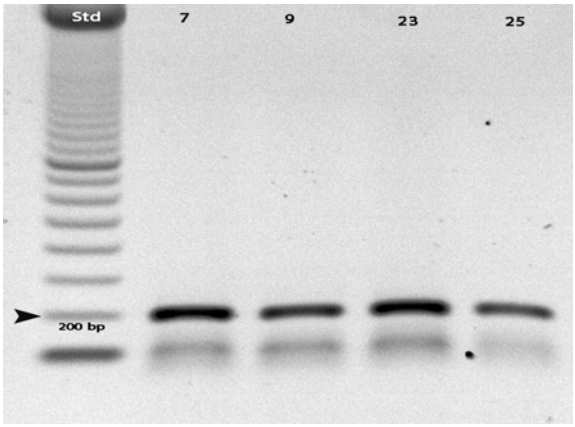

Figure 1: 1% agarose gel electrophoresis of Tubulin gene-specific RT-PCR products. "Std" lane is a 100 bp standard, and the Tub amplicons for samples 7, 9, 23, and 25 are in the expected size range of 200 bp.

Differential display PCR
A total of twenty eight differentially displayed bands were observed and excised from samples originating from diseased coral colonies (Figure 2).

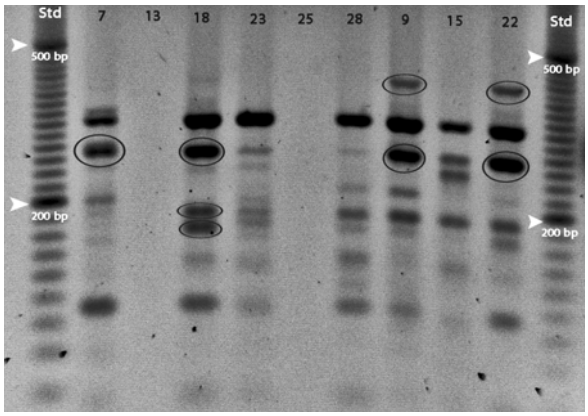

Figure 2: 2% agarose gel electrophoresis of DD-PCR products. Circled bands were excised for sequencing. Lanes "std" are standard 20 bp ladders, with white arrows indicating the 200 and 500 bp markers. The numbered lanes indicate the mucus samples from which the DD-PCR products were amplified.

Sequencing – programmed cell death gene detected at lesion boundary of White Plague-diseased corals

Sequences derived from the Cyt-B and Tub amplicons significantly matched ($E = 3e^{-30}$) sequences available for *D. strigosa* in NCBI. Sequences derived from differentially displayed bands amplified from a samples 7 and 18, originating from the lesion boundary of diseased colonies of *D. strigosa*, significantly matched ($E = 6e^{-9}$) a death domain sequence of *Nematostella vectensis* (Accession # XM 001639015.1).

Discussion

Coral innate immunity is not well understood, and to date, few molecular studies have been conducted to elucidate coral immune responses to disease (see Vollmer and Kline 2008 for a recent study). In this paper, results from a preliminary study on coral molecular responses to disease are discussed.

Most molecular analyses conducted on corals to date used invasive methods of collecting nucleic acids. These methods include the collection of entire coral colonies, large fragments or core samples that can reduce coral defenses on reefs that are already degraded (Edge et al. 2005, Smith-Keune and Dove 2007, Klueter *et al* 2006). The isolation of coral nucleic acids from condensed coral mucus is a novel method that can be used for PCR-based molecular analyses. The cellular sources of coral nucleic acids in the surface mucus layer of corals were not investigated in this study. However, mucocytes and nematocysts may release coral RNA and DNA into the surface mucus layer when the epidermis is stimulated. Amoebocytes, which are immunity-related motile cells that migrate throughout coral tissue (Meszaros and Bigger 1999), may also be shed into the mucus layer when physical irritation to the coral surface is applied.

Genomic DNA can compete with cDNA in PCR during primer annealing steps, and contamination of RNA samples with genomic DNA can significantly reduce the efficiency of DD-PCR to identify putative differentially expressed genes (Liang and Pardee 1995). Results from the negative control coral gene-specific RT-PCR reactions conducted in this study indicate a level of genomic DNA contamination undetectable by ethidium bromide staining. For that reason, an additional DNA digestion step was not performed, which would otherwise reduce the RNA concentration by a second precipitation step before cDNA synthesis.

The presence of Tub amplicons (200 bp) in all of the β-tubulin-specific RT-PCR reactions indicated that coral nucleic acids were present in each sample. However, a Cyt-B amplicon (500 bp) was not present for sample 13, which suggests that the sequence was degraded to a length significantly shorter than 500 bp. The collection of replicate mucus samples can ensure that at least one sample will have sufficient quality for DD-PCR. Variable intensity of DNA bands of the same base pair length in Figures 1 and 2 may show variable concentrations of coral RNA present in the samples. The number of coral cells and concentration of RNA excreted into the mucus may differ between diseased and healthy corals, and were unknown in this study. The starting concentration of total RNA used for reverse transcriptase reactions and DD-PCR can be normalized for samples from diseased and healthy corals, if an average concentration of coral RNA excreted into the mucus is determined by quantitative PCR amplification of the Cyt-B and Tub house-keeping genes.

Twenty eight differentially amplified bands were excised from DD-PCR products in this study. The sequencing of these amplicons is still underway, but preliminary results from the analysis of five candidate immunity genes have provided significant data upon which future investigations will be conducted. BLAST searches revealed sequence similarities of differentially amplified bands from samples 7 and 18 to a death domain sequence in *Nematostella vectensis*. Both of these samples were collected from the lesion boundary of *D. strigosa* colonies affected by putative White Plague infections.

Death domains are genes translated into conserved proteins that have functions in programmed cell death pathways (PCD) (Stehlik 2007). PCD has been investigated for its roles in mediating the destruction of cells in immune-compromised organisms, and it is activated by receptors such as Toll and Toll-like receptors (TLRs), which recognize conserved microbial ligands (Govind 2008, Ameisen 2002).

Gene sequences for TLRs have been identified in *Acropora millepora* and *N. vectensis* (Miller et al. 2007), and a recent study by Ainsworth and colleagues (2007) confirmed the presence of PCD in coral tissues adjacent to lesions characteristic of white syndromes on the Great Barrier Reef. The identification of a coral death domain as a putative immune response in *Diploria strigosa* colonies affected by putative White Plague infections suggests that PCD may have conserved functions in corals as a final line of defense against pathogenic microbial infections.

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Relative role of disease and predators as drivers of decline in coral cover on the Great Barrier Reef.

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Abstract. The Australian Institute of Marine Science monitored causes of coral mortality annually on 46 reefs throughout the GBR from 1999 to 2005 and a further 46 reefs biannually from 2006. Sampling consisted of categorising corals lesions according to signs associated with coral disease, crown-of-thorns starfish and *Drupella* spp. feeding activity and lesions that could not be categorized. Of those categories recorded only increases in crown-of-thorns starfish lesions were associated with subsequent declines in coral cover. Between 1999 and 2005 there was no clear evidence to suggest that disease outbreaks had a significant impact on live coral cover on survey reefs. This result is in contrast to those recorded from the Caribbean where coral disease has lead to wholesale declines in coral cover on affected reefs. The relative proportions of diseases recorded show that lesions due to white syndrome and unknown causes make up the majority of those observed. The direct causes of a majority of coral lesions remain unexplained highlighting the difficulty of classifying coral lesions based on visual signs.

Key words: coral, disease, crown-of-thorns, lesion

Introduction

Little is known about the role disease plays in shaping the coral reef community on the Great Barrier Reef (GBR). This is despite the first disease of reef building corals being described some forty years ago (Squires 1965). Populations off hard corals in parts of the Caribbean were decimated by disease during the late 1970's and 1980's (Gladfelter 1982, McClanahan and Muthiga 1998, Aronson and Precht 1997, 2001). This has been followed by an apparent increase in disease numbers recorded on the GBR during the 1990's (McClanahan et al. 2004; Willis et al. 2004; Miller et al. 2006).

Reductions in hard coral cover on reefs have a serious impact on ecosystem function and can result in "phase shifts" where ecological services provided by the ecosystem are radically changed as the reef community is altered from one dominated by hard corals to one dominated by fleshy macro algae (Hughes 1994). Despite the events in the Caribbean and the growing recognition of the role disease plays in the ecology of Indo-Pacific hard coral species (Willis et al. 2004; Winkler et al. 2004; Raymundo et al. 2005; Dalton and Smith 2006; Page and Willis 2008) there have been few reports of disease outbreaks affecting coral cover on the GBR (e.g. Jones et al. 2004). An increase in reports of disease in other parts of the world has coincided with an increased publication rate that confounds interpretation of any patterns over time (Ward and Lafferty 2004). To help interpret the role disease plays in shaping coral communities it is important to understand background levels of disease induced mortality (i.e. the rate of unavoidable mortality that is independent of the organisms' state or behavior McNamara et al. 2004), what constitutes an increase in disease incidence above these levels and subsequently what are the impacts (Willis et al. 2004; Raymundo et al. 2005). This is increasingly important for the conservation of the GBR, already threatened by crown-of-thorns starfish outbreaks (Sweatman 2008) and other threats resulting from global warming such as coral bleaching, more frequent and intense cyclones (Hughes 2003) and increased disease (Harvell et al. 1999. The Australian Institute of Marine Science (AIMS) Long Term Monitoring Program (LTMP) has been collecting information on disease and coral cover on fixed survey sites since 1999. We examine whether observed increases in disease activity are linked to declines in coral cover on surveyed reefs between 1999 and 2005. We also compare changes in coral cover to abundance of disease and other sources of mortality on the GBR in order to determine the relative impact of identifiable diseases.

Material and Methods

Surveys of benthic organisms and SCUBA searches categorising the putative causes of coral lesions (white bare skeleton indicating recent mortality) were conducted on 46 reefs annually from 1999 to 2005 and a further 46 reefs biannually from 2006 by the

AIMS LTMP. Reefs targeted for survey were located in six latitudinal sectors over a distance of approximately 1500 km along the continental shelf. Three shelf positions were sampled (inner, mid, and outer shelf) in the four northernmost sectors, two shelf positions (mid and outer) were sampled in the Swains sector, and one (outer) was sampled in the Capricorn Bunker sector (Fig. 1).

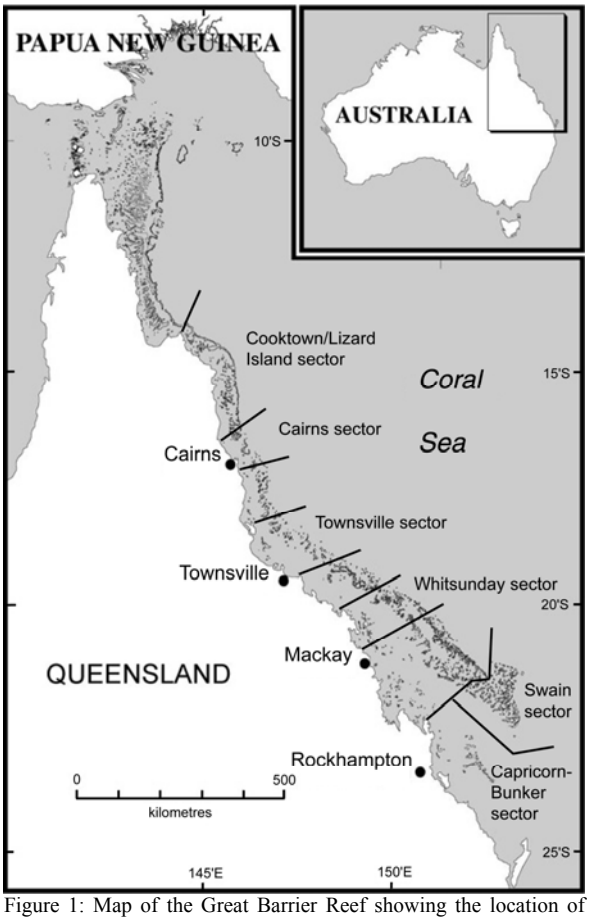

Figure 1: Map of the Great Barrier Reef showing the location of survey sectors targeted by the AIMS Long Term Monitoring Program.

At each reef, three sites positioned on the upper slope usually on the north-east flank were surveyed. Each site consisted of 5 x 50m transects along the 6-9m depth contour. Transects were marked with star pickets at the start and end, with steel rods at 10m intervals. Fibreglass measuring tapes attached to the pickets and rods allowed the same belt transect to be sampled in consecutive years.

A video recorder or still camera was used to record a 30cm wide strip along the edge of the measuring tape on each transect. Percent cover of hard corals was then estimated from the footage using a point-sampling technique. Images were captured from forty regularly-spaced frames of the video/still footage for each transect. Five systematically-distributed points were overlaid on each image and the identification of the benthic organism under each point was recorded. This provides a sample of 3000 points per reef. Hard and soft corals are identified to the highest taxonomic resolution possible, usually genus for hard coral and family for soft coral (see Abdo et al. 2004).

Causes of coral mortality were recorded using SCUBA searches conducted on a two-meter wide belt along each transect (search area $100m^2$). The observer recorded the number of individual lesions and ascribed their likely cause based on signs. These broadly fall into four major categories; disease including white syndrome (WS), black band disease (BBD), brown band disease (BrB), skeletal eroding band disease (SEB) and atramentous necrosis (AN); crown-of-thorns-starfish (COTS) ; *Drupella* spp. ; and unknown causes (see Miller 2004).

In 2006 a suite of 56 reefs (10 in common) were sampled using these methods as part of a separate research program (see Sweatman et al. 2008). The 46 LTMP reefs were again surveyed in 2007 but the time between surveys (2 years) meant that information collected in 2006 and 2007 could not be included in the statistical analysis as the model requires data collected in consecutive years. However, to determine the current relative frequency of disease types on the GBR a breakdown of the average number off disease lesions attributable to each of the categories recorded on all reefs over the period 2005 and 2007 was calculated.

To explore the relationship between changes in coral cover and coral lesion abundance a General-Linear-Model was used with log transformed coral cover as the response variable, log coral cover in the previous year as the main predictor and incidence of diseases as covariates. In log-space this kind of regression model is equivalent to the Gompertz growth model, a deterministic model frequently used to describe density-dependent population growth in discrete-time (Reddingius 1971; Dennis and Taper 1994). The intercept of the regression model estimates the log of the intrinsic growth rate, the coefficient of the population in the previous time period gives the strength of density dependence, and the coefficients of the covariates are modifiers of the growth rate and estimate the log of the proportional impact they have on population size.

Covariates (impacts) tested included the average number of lesions recorded between 1999 and 2005 for each of disease (pooled); *Drupella* spp.; COTS; and unknown causes. Covariates were square root transformed prior to analysis. All data were entered into an initial regression model with all covariates. Standardized residuals values were plotted against number of lesions due to COTS, unknown causes, disease and *Drupella* spp.. Outliers were identified

and removed for reefs where severe COTS outbreaks had decimated coral cover in the previous year, and where observed declines were due to mass coral bleaching in 1998 and cyclone damage on Low Isles reef also in 1998. A backward stepwise variable selection procedure was then used to test the importance of covariates. Covariates with the smallest partial correlation with the dependent variable were sequentially removed until all remaining covariates failed the removal criteria (probability of F-to-remove >= 0.10).

Results

Covariates for unknown causes, disease and *Drupella* spp. were all eliminated by the backward stepwise regression modeling procedure (Table 1) indicating that there was no significant association ($p > 0.10$) between coral cover in the subsequent year and lesions caused by these agents. There was, however a significant negative relationship between coral cover in subsequent years and increased COTS lesion abundance. COTS lesions were retained as a covariate in the final model (Table 2).

Covariate removed	Df1	Df2	Change in F	p (F)
Disease lesions	1	260	0.010	0.921
Drupella spp. lesions	1	261	1.256	0.263
Unclassified lesions	1	262	0.953	0.330

Table 1: Covariates eliminated during backward stepwise regression procedure and their corresponding changes in F-statistic

Covariate	Estimate	Std. error	t	P
Constant	0.224	0.073	3.09	0.002
Initial cover	0.939	0.021	44.1	<0.001
COTS lesions	-0.206	0.018	-11.6	<0.001

Table 2: Final regression model resulting from backwards stepwise regression procedure. The constant estimates the log of the intrinsic growth rate and the initial cover parameter estimates the strength of density dependence. Together they define the equilibrium coral cover [exp (0.224 / (1-0.939) = 39.3%]. The COTS lesions parameter estimates the proportional impact on next year's coral cover of COTS outbreaks that result in √x observed COTS lesions [exp (-0.206) = 0.81].

The majority of lesions recorded on fixed transects between 2005 and 2007 could not be assigned a cause (Fig. 2). White syndrome and *Drupella* spp. lesions were the most commonly assigned while lesions due to black band disease and atramentous necrosis were the least assigned. Only a small proportion (6.3 ± 1%) of the lesions observed could be attributed to COTS, which were on average less common than lesions due to *Drupella* spp., WS or SEB. There was some regional variation in the prevalence of lesion types. While unknown lesions dominate the counts in the majority of sectors WS was particularly common in the Capricorn Bunker sector. SEB was most common on northern reefs in the Cooktown Lizard Island sector while BrB was highest in the Pompey sector. BBD and AN were all observed at very low levels in the majority of sectors surveyed.

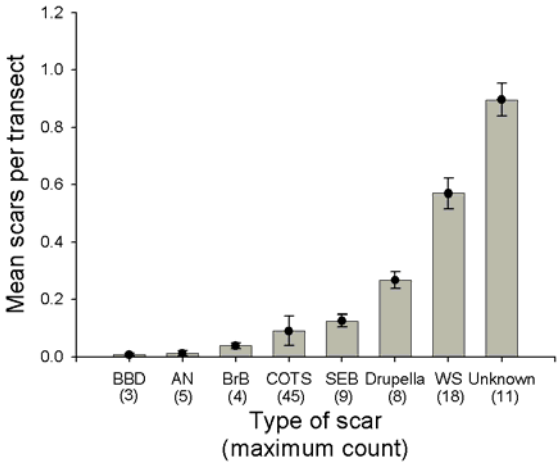

Figure 2: Abundance (mean ± SE) of different coral lesion types recorded per transect during AIMS scuba search surveys on fixed sites recorded from 2005-2007.

Discussion

The major finding of this study was the absence of a relationship between coral cover and the abundance of disease lesions on reefs of the GBR. In fact of all the factors tested only increases in lesion abundance due to COTS predation was positively associated with subsequent declines in coral cover. The latter result is not surprising as COTS have long been known to be a major source of coral mortality since outbreaks were first indentified in the 1960's (Birkeland and Lucas 1990). On the GBR three series of COTS outbreaks have caused declines in coral cover that have dwarfed losses from other disturbances such as storms or coral bleaching recorded over the same period (Sweatman et al. 2000). That there is no clear relationship between abundance of disease lesions and subsequent changes in coral cover on the GBR is in direct contrast to many other studies, particularly those in the Caribbean. In the Caribbean the most abundant reef building corals (*Acropora* spp.), were decimated by epizootics during the 1970's and early 1980's (Gladfelter 1982, McClanahan and Muthiga 1998, Aronson and Precht 1997). Since then coral cover on many reefs in the region has continued to decline with more epizootics recorded in recent times (Porter et al 2001, Patterson et al. 2002). While epizootics have been recorded from the GBR (Jones et al. 2004) they are rare. In fact reports of disease from the Indo-

Pacific, in general make up a much smaller proportion of reports when compared to those from the Caribbean (Willis et. al. 2004). This is despite the fact that the Indo-Pacific region is home to more than 80% of the reefs worldwide (Bryant et al. 1998). A possible explanation for the GBR is that coral diseases in this region are less virulent than in the Caribbean. Over the period 1977 to 2002 Gardner et al. (2003) reported a region-wide decline in hard coral cover in the Caribbean basin of 80% with coral disease playing a key role in this decline. Conversely declines in coral cover on the GBR over the last two decades have been generally driven by factors other than disease and where declines in some regions have been recorded they have been balanced by increases in others (Sweatman et al. 2008). In the Caribbean coral disease can have devastating affects on coral populations even when coral cover is low. For instance between 1996 and 1999 losses of *Acropora palmata* in the Florida Keys national Marine Sanctuary averaged 87% or greater from an already low (12% on average) cover (Sutherland 2004). In contrast on the GBR coral cover needs to be over 50% before outbreaks of white syndrome can occur (Bruno et. al 2007).

Another explanation for the observed result is that extrinsic factors may be differentially driving increases in the prevalence of coral disease in the Caribbean compared to those recorded from the GBR. However while many studies have pointed to rising disease prevalence (Ben-Haim and Rosenberg 2002, Sutherland et al. 2004, Weil et al. 2006), other studies have not always supported these conclusions (Voss and Richardson 2006) and a lack of baseline studies makes any interpretation of recent trends difficult (Ward and Lafferty 2004).

A third possible explanation is that lesions have been recorded at a level where they play a limited role driving observed changes in coral cover and that disease induced mortality on the GBR is simply outweighed by growth and renewal i.e. "background" levels of coral mortality (McNamara et al. 2004).

Previous studies have highlighted the increasing number of hard coral disease recognized on the GBR (Willis et al. 2004). Surveys by the AIMS LTMP in recent years have endeavored to include emerging diseases once signs for their identification have been confirmed. As a result the LTMP provides a clear picture of the ubiquity of coral disease on the GBR. Coral diseases appear widespread on the GBR with lesions caused by WS and SEB generally more frequent than those encountered for either *Drupella* spp. or COTS. This highlights that coral mortality due to disease, in particular SEB and WS, is a common occurrence on the GBR. All five disease types recorded by the LTMP have been generally found throughout the GBR system (AIMS data) though BBD, BrB and AN remain relatively rare. White syndrome is the most commonly encountered disease on the GBR. This is in contrast to other research that shows SEB to be generally more common than other diseases (Page and Willis 2008). The reason(s) for the apparently different results are unclear. Both WS and SEB have been well described with a known cause (Sussman et al. 2008, Antonius 1999). A possible explanation is that the divergent results are at least partially due to differences in sampling technique (i.e. observers, different locations on the reef surveyed, different survey methods including search area and time). Furthermore the most abundant lesion category recorded during this study was for those that remain unidentified. This highlights the difficulty of identifying signs of disease in the field. The problems of characterizing a disease (Richardson 1998) and then using the external appearance of corals for diagnosis is well recognized (Ainsworth et al. 2007a). The AIMS LTMP overcomes this problem by having a clear methodology (Miller 2004).

In terms of future scenarios for coral epizootics on the GBR indentifying disease and understanding disease dynamics, including trends in disease occurrence, is fundamental to conserving ecosystems faced with rising anthropogenic stresses (Ward and Lafferty 2004). This is particularly true given the relatively pristine nature of the GBR and that it is already under threat from COTS (Sweatman 2008), coral bleaching (Berkelmans et. al. 2004) and increased frequency and intensity of cyclones (Hughes 2003). The need to identify disease in the field and understand its role in the reef community can only become more important as increases in water temperatures (Jones et al. 2004, Bruno et al. 2007, Bourne 2008), nutrients (Bruno et al. 2003, Voss and Richardson 2006) bleaching (Muller et al. 2008) and in some cases, interactions with other organisms (Nugues et al. 2004, Aeby 2007, Nugues and Bak 2009) may be expected lead to increased prevalence of disease on the GBR in future years.

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Significance of immune responses in *Diadema antillarum* to Caribbean-wide mass mortality

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Abstract. Mass mortality of the grazer *Diadema antillarum*, probably caused by a water-borne pathogen, was a major factor leading to algal domination of Caribbean reefs. Recovery of *Diadema* in St. Croix allows us to addresses questions on how recovery may be influenced by immunological processes. What are the basic features of immunological responses in *Diadema*? How does strength of immune responses in *Diadema* compare to other urchins that did not die (*e.g., E. lucunter*, or *E. viridis*)? We used coelomic fluid and coelomocytes of urchins from several locations on St. Croix to test their immune responses. All urchins released humoral defense molecules when stimulated by various agents – with one exception. *Diadema* did not respond as efficiently as other urchins did to lipopolysaccharide. This suggests a defect in immune response that is specific to *Diadema* and independent of stressors associated with particular environments. These studies may provide information required to understand whether a weakened immune response was responsible for the mass mortality, and whether strengthening of immunity has occurred since. Effective management of a recovery may depend on knowing whether diseases continue to impact *Diadema* population growth due to weak immune responses, and whether a recovered/restored population could experience another die-off.

Keywords: Comparative Immunology, Echinoderm, Ecology, Evolution, Immunity

Introduction

The Black-spined Sea Urchin and Coral Reef Ecology

In 1983 the black-spined sea urchin (*Diadema antillarum*) began to vanish from the Caribbean Sea, disappearing first from coral reefs close to the Panama Canal and eventually from almost every coral reef in the Caribbean by 1984. This mass mortality, which wiped out more than 97% of the Caribbean-wide *Diadema* population, was one of the most devastating mortalities ever recorded for a marine animal. This die-off is a major factor leading to a phase shift from coral-dominated to algae-dominated communities that has occurred on many Caribbean reefs during the past 20 years (Lessios 1988; Hughes 1994). In St. Croix, the density of *Diadema* before the 1983 die-off was estimated to be ≈6.4 individuals/m^2. In 1984, densities plummeted to <0.1 individuals/m^2, and in 2001 there were ≈0.17 individuals/m^2 (Lessios 1988). Decades after the mass mortality event, *Diadema* was still rare, with very low recruitment rates (Lessios 1988; Edmunds and Carpenter 2001).

It is thought that the demise of *Diadema* was caused by a water-borne pathogen. Indeed, plots of surface currents in the Caribbean Sea coincide significantly with the spread of the *Diadema* die-off (Lessios 1988; Miller et al. 2003). In addition, several species of bacteria capable of killing *Diadema* have been associated with dying urchins in the laboratory, but bacteria have not been detected in field-collected individuals (Bauer and Agerter 1987).

Recent recovery of *Diadema* has been associated with reductions in algal cover and increased coral recruitment success in Jamaica (Edmunds and Carpenter 2001), and with reductions in algal cover in St. Croix (Miller et al. 2003), where *Acropora palmata* colonies are reappearing in areas with high *Diadema* densities, but are largely absent where *Diadema* are absent or uncommon (unpublished). Recovery of *Diadema* may be critical to restoration of coral communities, particularly where reefs have few herbivorous fishes due to heavy fishing (Hay 1984), as in the USVI (Garrison et al. 1998). Many factors may be influencing *Diadema* recovery, including predation, competition from other herbivores, and effects of rarity [low reproduction and recruitment stemming from low population density (*e.g.,* Lessios 1988; Miller et al. 2003)]. Another factor that must be considered is possible immunological weakness of this *Diadema* population which would allow continuing morbidity or mortality due to the original mass mortality pathogen or other diseases. Continuing recovery of *Diadema* populations in St. Croix (Miller et al. 2003) has allowed study of how recovery dynamics of *Diadema* may be influenced by basic

immunological and ecological processes.

Our studies are aimed at uncovering basic features of the immunological responses of *Diadema*: What are the basic features of the immunological responses of *Diadema*? How does strength of immune responses in *Diadema* compare to other Caribbean urchins that did not die off (*e.g., Tripneustes ventricosa, Echinometra lucunter,* or *Echinometra viridis*)? Are there immunological differences in local populations of *Diadema* on St. Croix that could be related to site-specific speeds of recovery?

Invertebrate Immunity

Animals posses both cellular and secreted protein (humoral) mechanisms for distinguishing self from non-self. Potential invaders include viruses, bacteria, protozoa, fungi and even protein molecules. The distinction between "self" and "nonself" is achieved by even the most primitive protozoans (Beck and Habicht 1996; Flajnik and DuPasquier 2004). Evolutionary forces have given rise to two arms of the immune system in animals: acquired (also known as adaptive or specific) and innate (or non-specific) immunity. Acquired immunity is only found in vertebrates. Invertebrates have innate immune systems, characterized by activation of immune effector cells, mediation by circulating coelomocytes. Invertebrates have many of the innate host defense mechanisms vertebrates do, but lack the specificity of the vertebrate immune response. The position in phylogeny of echinoderms makes them an important invertebrate group for comparative studies, including those addressing the evolution of the immune system. Although echinoderms efficiently clear bacteria from the coelomic fluid (CF) and mount an innate type immune response to pathogens and their products by coelomic fluid cells (coelomocytes), little is understood of the molecular mechanisms and genes that are used by these cells in response to a non-self challenge.

Phagocytosis [the engulfment of foreign material (*i.e*., pathogens) by specialized cells (*i.e*., phagocytes)] is the predominant cellular defense mechanism in vertebrates and invertebrates (Beck and Habicht 1996; Flajnik and DuPasquier 2004). Humoral-based defenses include: antimicrobial peptides (AMPs) and proteins (*i.e.,* lysozyme), phenoloxidase, complement-like proteins (Beck and Habicht 1996; Flajnik and DuPasquier 2004; Raftos et al. 1992; Beck et al. 1996; Lin et al. 2001), and reactive oxygen intermediates [ROI; *e.g.*, superoxide ($O_2^{\cdot-}$)] (Meier 2001; Babior et al. 1973; Clifford and Repine 1984; Beck et al. 2001). The ability of invertebrates to combat infections is dependent on the release of ROI (a group of inorganic compounds that are used by phagocytes in the formation of more toxic radicals to destroy pathogens).

Materials and Methods

Reef surveys: At each back-reef site at each sampling time, SCUBA divers counted *Diadema* in 14 randomly located 50m × 2m transects, and *Diadema* test diameters were recorded as small [<40mm; mean size of 1 yr. old *Diadema* = 48.6 mm (Karlson and Levitan 1990)], medium (41-60mm) or large (>60mm).

Collection of sea urchin coelomocytes: Urchins were collected from three study sites around St. Croix and processed within 2 hrs. A syringe containing ice-cold anticoagulant solution (99.8g EDTA and 33.5 ml 2-mercaptoethanol to 1L in seawater) was inserted into the coelom through the soft tissue surrounding the urchin mouth structure (Aristotle's lantern) to extract coelomocytes (Beck and Habicht 1991). These cells were washed two times and resuspended in saline at a concentration of 1×10^6 cells/ml. Cells were counted in a hemocytometer (Lin and Beck 2007). Proteins in the CF were quantified by the Bradford assay (Beck and Habicht 1991; Lin and Beck 2007). Sodium dodecycl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the CF was performed as previously described (Lin et al. 2001; Beck and Habicht 1991).

Determination of reactive oxygen intermediates (ROIs): The ROIs produced by phagocytes were assayed using a spectrophotometric assay to measure $O_2^{\cdot-}$. When phagocytes engulf a pathogen, a burst in oxidative metabolism results in the generation of ROIs (Beck et al 2001; Arumugam et al. 2000; Anderson et al. 1992; Vidya et al. 2007; Bettencourt et al. 2007). A simple and direct method of detecting this metabolic change is the nitro-blue tetrazolium (NBT) reduction assay [wherein the yellowish NBT is reduced to an insoluble, purplish precipitate in the cells (Arumugam et al. 2000; Anderson et al. 1992)]. Two hundred fifty µl cells (10^6 cells/ml), 250 µl of 0.1% NBT, 10µl stimulator [*e.g.,* peptidoglycan, lipopolysaccharide in phosphate buffered saline (PBS)]) or PBS alone (control) were mixed and incubated at 22ºC. (Beck et al 2001; Arumugam et al. 2000; Anderson et al. 1992; Vidya et al. 2007; Bettencourt et al. 2007). After 1 hr the cells were separated from the supernatant by centrifugation in a microfuge, and reduced NBT in the pellet was determined spectrophotometrically at an optical density at 630 nm (in quadruplicate). The production of $O_2^{\cdot-}$ was monitored over time.

Results

Enumeration of Diadema on the Reefs of St. Croix

We asked whether the initial recovery of *Diadema* that we have observed on critically affected coral reefs of St. Croix (Miller et al. 2003) is continuing? To determine relative rates of population recovery in different parts of St. Croix, U.S.V.I., *Diadema* were counted and sized twice at each of six locations (Fig. 1): Two south shore back-reef sites (TH & RB), two north shore back-reef sites (POW & SOL), and two north shore patch reefs in Tague Bay (TB), following the methods of Miller *et al.* (Miller et al. 2003). As can be seen in Fig. 2, recovery of *Diadema* on St. Croix continues to be slow, or has stalled out entirely.

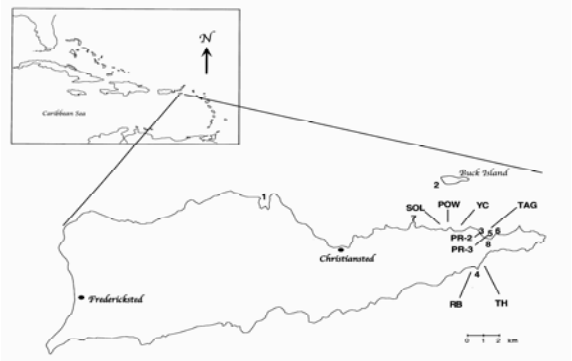

Figure 1: Map of St. Croix, USVI showing the 6 study sites. SOL = Solitude Bay, POW = Pow Point, YC = Yellowcliff Bay, TAG = Tague Bay, TH = Turner Hole, RB = Rod Bay. (From 4)

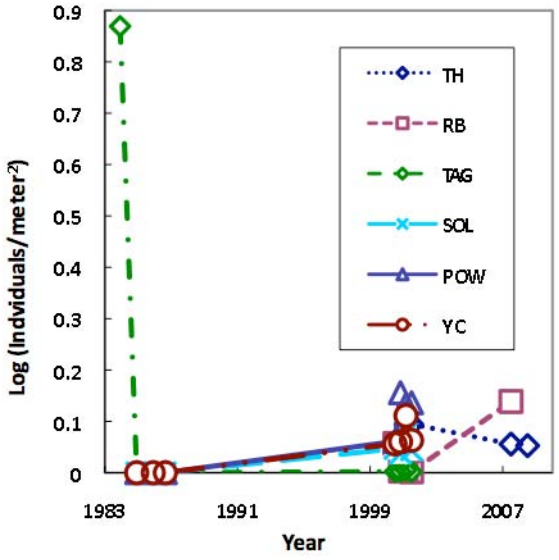

Figure 2: The number of *Diadema* found at study sites on St. Croix from 1984 to 2008. The study sites are the same as described in Fig. 1.

Gross Protein Profiles and Cellular Components of Coelomic Fluid in the Urchins

Protein profiles and cellular components of the coelomic fluid varied substantially among the four urchin species (Fig. 3). *Diadema* differs significantly from *Tripneustes* and the two *Echinometra* species in the counts for both red blood cells and total coelomic cells, but in both cases the values for *Diadema* are in the middle of the pack and not at either extreme. On the other hand, as determined by the Bradford assay, *Diadema* has significantly more protein in the CF than any of the other urchins.

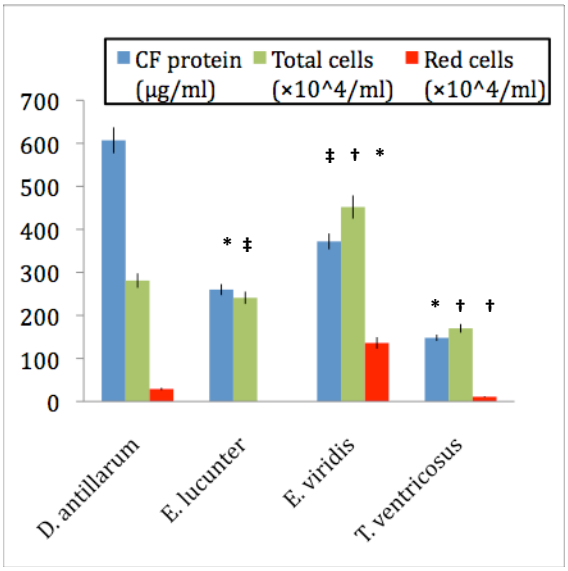

Figure 3: Analysis of CF and coelomocytes of 4 urchins found in the coastal waters of St. Croix (mean ± SD for 6 urchins of each species). **P*< 0.001 when compared to *Diadema* alone, †*P*< 0.01 when compared to *Diadema* alone, ‡*P*< 0.05 when compared to *Diadema* alone.

Differences in Protein Profiles of Coelomic Fluids of Diadema from Different Sites (SDS-PAGE)

When CF proteins from *Diadema* collected from 3 different sites on St. Croix (SOL, RB, and TH) were subjected to SDS-PAGE we could see no readily apparent differences in their crude protein profile (Fig. 4).

Changes in the Humoral Responses of the Urchins

The ability of invertebrates to combat infections depends, in large part, on the release of reactive oxygen intermediates, groups of inorganic compounds that inhibit vital enzymes of pathogens and are used by phagocytes in the formation of more toxic radicals (Arumugam et al. 2000; Anderson et al. 1992; Vidya

et al. 2007; Bettencourt et al. 2007). As seen in Fig. 5 all the urchins were capable of releasing $O_2^{\cdot-}$ when stimulated by various agents. There was one statistically significant exception: The response of *Diadema* to stimulation with lipopolysaccharide (LPS) is low compared to the other urchins. There is no geographic component to the lack of this response to LPS since *Diadema* from all sites were equally unresponsive (unpublished).

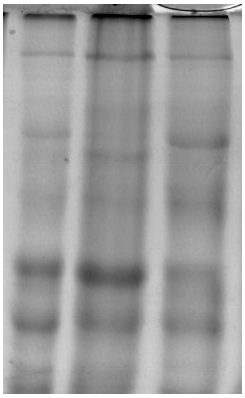

Figure 4: SDS-PAGE (7.5% SDS-PAGE gel stained with Coomassie Blue R-250) of CF from *Diadema* collected from 3 different locations on St. Croix.

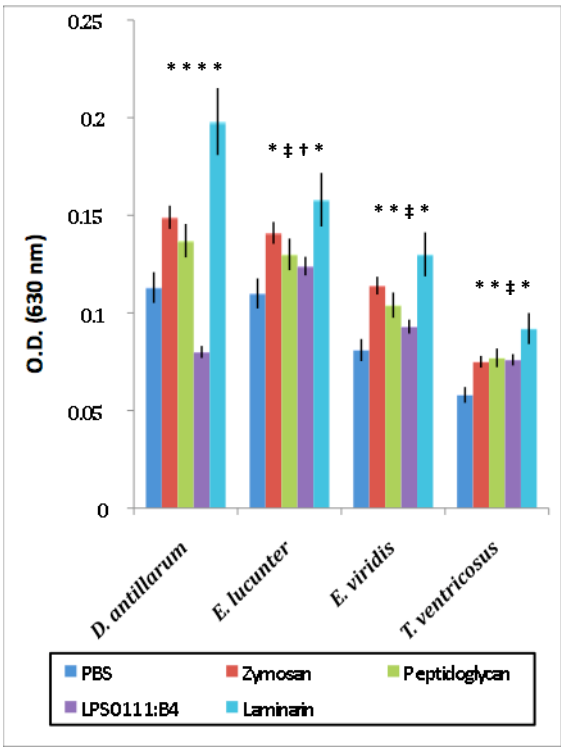

Figure 5: Stimulation of sea urchin coelomocytes with different agents. Data are presented as mean ± SD from six experiments. *P< 0.001 when compared to PBS control, †P< 0.01 when compared to PBS, ‡P< 0.05 when compared to PBS.

Conclusions

Recovery of *Diadema* on St. Croix continues to be slow, or has stalled out entirely. This recovery, with its beneficial effects on corals, is neither universal nor complete in the Caribbeanmand is progressing even more poorly in the Florida Keys (Miller et al. 2003; Carpenter and Edmunds 2006; personal observation). A principle recommendation of the recent *Diadema* Workshop, hosted by the Nature Conservancy, the National Fish and Wildlife Foundation, and the Rosenstiel School of Marine and Atmospheric Sciences in the summer of 2004, was to study key aspects of *Diadema* biology and ecology. Efforts to restore *Diadema* in the Florida Keys by seeding small areas with juvenile urchins have been unsuccessful because juveniles suffered severe mortality after they were introduced to the reefs (Rosov 2002). Efforts to reverse the algae-coral phase shift on coral reefs of the Caribbean and Florida Keys through restoration/recovery of *Diadema antillarum* can succeed only through improved comprehension of the recovery process. Managing a more rapid recovery may depend on understanding the competitive and predator/prey relationships between fishes and urchins, and how increasing adult *Diadema* densities affect recruitment and juvenile survival. It is also critical to understand whether weakened immune systems in *Diadema* were partly responsible for the mass mortality, and how much strengthening of immune systems has occurred since the die-off.

We used a preliminary broad-based "shotgun" approach (*e.g.,* coelomic fluid and coelomocyte population characterization) in an attempt to quantify any differences in host defense responses between several Caribbean sea urchin species. Our experiments suggest that the strength of the humoral immune response in *Diadema* appears to be weaker than in other urchin species of St. Croix. In addition, in both humoral assays and SDS-PAGE experiments we have observed that there are no differences among *Diadema* from different St. Croix locations. The inability of *Diadema* to respond as efficiently to LPS –a conserved structural component of gram-negative bacterial cell walls– as other urchins do could be a clue to a defect in a host defense response to a pathogen. One possible explanation for this decreased responsiveness could be a defect in Toll-like receptors (TLRs). The TLRs are a family of essential cell surface proteins of the innate immune system of vertebrates and invertebrates (Bochud et al. 2007; Akira et al. 2006; Beutler 2004). Their extracellular domains are capable of recognition of different microbial-associated molecular patterns (MAMPS). For example, TLR-4 detects LPS and mannan, while TLR-2 detects peptidoglycan and other β-glycans

(Bochud et al. 2007; Akira et al. 2006; Beutler 2004). This apparent *Diadema* lower response to LPS could be due to a defect in TLR-4 or a second polypeptide (MD-2) that binds to the extracellular domain of TLR-4 and enables it to bind to the conserved inner region of LPS. Is there a defect in LPS signaling in *Diadema* [*e.g.,* TLR-4 and/or MD-2 defects, a TLR-4/MD-2 signaling defect)? One could argue that not all *Diadema* TLRs are defective as *Diadema* responds appropriately (when compared to the other urchins) to peptidoglycan and zymosan (TLR-2 ligands) (Fig. 5).

Whatever the molecular basis for a weakened immune response in *Diadema antillarum*, the weakness could have consequences for the future of Caribbean coral reefs. The recovery dynamics of *Diadema* have been patchy in St. Croix waters, with some localities experiencing earlier, and/or more rapid recovery than others. Since *Diadema* from all parts of St. Croix seem to be showing the same degree of immunological weakness, we cannot associate local differences in recovery with local differences in immune response. Efforts to reverse the algae-coral phase shift on coral reefs of the Caribbean and Florida Keys through restoration/recovery of *Diadema* can succeed only through improved comprehension of the recovery process. It is critical to understand whether a weakened immune response in *Diadema* was responsible for the mass mortality, how much strengthening (if any) of immune systems has occurred since, whether continuing weakness may be interfering with *Diadema* recovery, and whether a recovered population could experience another epidemic. However slow, continuing recovery of Caribbean *Diadema* and the existence of populations of *Diadema antillarum* and related species that were not involved in the die-off affords an opportunity to look at how recovery and post-recovery dynamics of *Diadema* may be influenced by the development of immunological resistance.

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The future of coral reefs in the US Virgin Islands: Is *Acropora palmata* more likely to recover than *Montastraea annularis* complex?

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Abstract. Coral diseases have played a major role in the degradation of coral reefs in the Caribbean, including those in the US Virgin Islands (USVI). In 2005, bleaching affected reefs throughout the Caribbean, and was especially severe on USVI reefs. Some corals began to regain their color as water temperatures cooled, but an outbreak of disease (primarily white plague) led to losses of over 60% of the total live coral cover. *Montastraea annularis*, the most abundant coral, was disproportionately affected, and decreased in relative abundance. The threatened species *Acropora palmata* bleached for the first time on record in the USVI but suffered less bleaching and less mortality from disease than *M. annularis*. *Acropora palmata* and *M. annularis* are the two most significant species in the USVI because of their structural role in the architecture of the reefs, the large size of their colonies, and their complex morphology. The future of the USVI reefs depends largely on their fate. *Acropora palmata* is more likely to recover than *M. annularis* for many reasons, including its faster growth rate, and its lower vulnerability to bleaching and disease.

Key words: disease, *Acropora palmata, Montastraea annularis*

Introduction

Coral diseases have played a major role in the degradation of coral reefs in the Caribbean, including those in the US Virgin Islands (USVI) (Weil et al. 2006, Rogers et al. 2008a). White band disease, first described from St. Croix, USVI (Gladfelter 1982), decimated *Acropora palmata* in the late 1970s and 1980s (Gladfelter 1982, Aronson and Precht 2001). Now there are many more coral diseases, and they are affecting virtually all coral species (Weil 2004). White plague has been the primary disease affecting *Montastraea annularis* and other massive corals. Although white band disease is less common now in the USVI, white pox disease and other un-described diseases are affecting *A. palmata*. In this paper we use the terms white pox and white plague based on the similarity of the gross signs observed in the field to descriptions in the scientific literature. These diseases have been associated with the pathogens *Serratia marcescens* (Patterson et al. 2002) and *Aurantimonas coralicida (*Denner et al. 2003), respectively, but these bacteria have not consistently been isolated from diseased corals at USVI (or other) study sites (Polson 2007). As Work et al. (2008) and others have noted, coral disease investigations are at a very early stage.

Although the coral reefs in the USVI have deteriorated from overfishing, runoff, hurricanes, and a number of other causes (Rogers and Beets 2001, Rogers et al. 2008a, Rothenberger et al. 2008), the greatest losses since studies began over 40 years ago have been from the combination of bleaching and disease from 2005 to 2006 (Miller et al. 2006, Muller et al. 2008, Rogers et al. 2008b). Conclusions that 18% of the world's coral reefs will likely be "lost" by 2030 (IPCC 2007) are appearing in the scientific literature. Although there is no clarification of just what is meant by the word "lost", many reefs have certainly deteriorated severely. Specifically, in the Caribbean, live coral cover has declined significantly, and primary reef-building species such as *Acropora palmata* and *Montastraea annularis* have decreased in abundance.

Here we briefly describe the recent declines in the USVI and discuss the potential for recovery of the two most significant species, *A. palmata* and *M. annularis*. *Montastraea annularis* is the most abundant coral on deeper (5 to 20 m) reefs, outside the *A. palmata* zones. These species are the main reef builders, with colonies that can reach several meters across, and with a complex morphology that provides shelter and habitat for a diversity of fish and other

organisms (Goreau 1959, Shinn 1963, Ogden and Ehrlich 1977, Lirman 1999).

Effects of bleaching and disease on deeper (5 to 20 m) reefs in the USVI

The Caribbean-wide bleaching event in 2005 and subsequent disease outbreak led to an average loss of over 50% of the living coral cover at long-term study sites in the USVI in one year and an average loss of over 60% in two years (Rogers et al. 2008b). Four of the five sites are within Marine Protected Areas [Virgin Islands National Park (St. John) and Buck Island Reef National Monument (St. Croix)]. These are being monitored by National Park Service (NPS) biologists with the South Florida Caribbean Inventory & Monitoring Network. An average of c. 96% of the total coral cover bleached, including over 90% of the *Montastraea annularis* complex. [*Montastraea annularis* complex, abbreviated as *Mac*x, refers to *M. annularis*, *M. franksi*, and *M. faveolata* (Weil and Knowlton 1994)]. Over 90% of the disease lesions and the area killed by disease occurred on *Mac*x, and the relative abundance of *Mac*x went from 79.2% to 58.6%. *Montastraea annularis* initially made up 55.6% of the coral cover but declined to 40.9% by 2007. The total coral cover on these reefs fell from an average of 21.4% to 10.3% after 1 yr and then to 8.3% after 2 yrs. Because National Park Service and US Geological Survey scientists increased the frequency of monitoring to every few months during the bleaching event and subsequent disease outbreak, it was clear that the significant coral mortality was from disease, not directly from bleaching.

Effects of bleaching and disease on *Acropora palmata* colonies

USGS biologists were monitoring individual *A. palmata* colonies during this event and observed bleaching of this species for the first time on record in the USVI. About 48% (of a subset of 467 colonies that were being monitored) bleached, 13% died partially, and only 8% died completely. Muller et al. (2008) found a positive relationship between temperature and disease in 2005, the year of the bleaching event. In addition, the average area of disease-associated mortality was significantly higher on bleached colonies, suggesting that disease severity was related to bleaching susceptibility. Although some colonies died partly or totally from bleaching and disease, this species did not suffer such extensive losses as *M. annularis* and many other species. The bleached *A. palmata* colonies around St. John had regained their normal color by January 2006. In contrast, *M. annularis* colonies at the long-term study reefs remained pale until at least October 2006.

Potential for recovery of *Acropora palmata* and *Montastraea annularis*

For a variety of reasons, *A. palmata* has a greater chance of recovering than *M. annularis*. What exactly is meant by recovery? In the strictest sense, full recovery would entail a return to the same number of colonies, overall amount of living coral cover, and genotypic diversity present at a specified time. Currently *M. annularis* is found to depths over 40 m (Armstrong et al. 2006) and in several reef zones, and is far more abundant than *Acropora palmata* which occurs typically in less than 8 m and in fewer zones. Even before the 2005/2006 bleaching and disease event, Edmunds and Elahi (2007) predicted possible extirpation of *M. annularis* at one site in St. John, based on a demographic model using data from three 10-m long transects dominated by this species.

More information is needed on the levels of sexual recruitment in both species. Both species are broadcast spawners and both spawn in late summer, a time of higher vulnerability because of hurricanes and generally warmer water. Several studies show low levels of recruitment for both species (Bak and Engel 1979, Rogers et al. 1984, Edmunds 2000). However, the genotypic diversity of *A. palmata* around St. John is quite high and is evidence that sexual recruitment is occurring here as well as elsewhere in the Caribbean (Baums et al. 2006, Rogers et al. 2008b; but see Williams et al. 2008). For example, in Haulover Bay, 43 of 48 colonies were different genotypes. Sexual recruits of *A. palmata* on dead portions of *A. palmata* colonies cannot be distinguished from surviving remnants, but around St. John many small colonies growing directly on rock substrate are further evidence of sexual recruitment. Genotypic diversity of *Mac*x is unknown in this area and certainly a confounding factor when evaluating the potential for recovery.

Although both *A. palmata* and *M. annularis* are able to reproduce asexually (Highsmith 1982, Foster et al. 2007), *A. palmata* as a branching species probably has a greater potential for dispersal through fragmentation. This species typically has more damage from storms than massive colonies (Woodley et al. 1981, Hubbard et al. 1991). The columnar or lobar morphology of *M. annularis* begins to develop when the colony is still relatively small, in some cases less than 30 cm across. Although the separate lobes can be dislodged and dispersed, fragmentation is not as likely as in *A. palmata* and the separate "fragments" are heavier and probably not often transported far from the donor colony. (Unlike *A. palmata* fragments, the lobes often separate from the colony at the base where there is coral skeleton, not

living coral.) Of course, not all *A. palmata* fragments attach and survive (Lirman 2000a, Williams et al. 2008). At Saltpond Bay, on the south side of St. John, 60% of the (naturally-generated) fragments survived for three or more months (USGS, unpublished data).

A few recent studies use analysis of genotypes to document dispersal of clones. Baums et al. (2006) showed that *A. palmata* fragments from one genet had dispersed over a distance of more than 70 m on one Florida reef. Foster et al. (2007) reported dispersal of *M. annularis* lobes (columns) up to 9 m away on Honduras reefs affected by storms, but the clonality was lower than reported for many *A. palmata* populations (Baums et al. 2006).

The scientific literature has few studies of growth rates of corals from different locations. However, in the USVI *A. palmata* has much higher growth rates (about 5 to 10 cm per year, Gladfelter et al. 1978) than *M. annularis* (less than 1 cm per year, Gladfelter et al. 1978, Hubbard and Scaturo 1985).

Healing rates of *A. palmata* after physical damage, for example from storms, and regeneration of disease lesions can be quite rapid (Rogers et al. 1982, Lirman 2000b, Muller 2007). Less is known about the ability for lesions on *M. annularis* to regenerate, and the nature of the damage to this species differs in that colonies are often abraded by sand or dislodged from their bases rather than fractured. The cause of the lesions can make a difference in the coral's ability to regenerate tissue or tissue and skeleton. Meesters et al. (1994) noted that experimentally-created lesions on *M. annularis* usually did not completely close, leaving bare space for colonization by algae and other organisms. Bak et al. (1977) showed repair of small, experimentally-created lesions on *M. annularis* over several days. Bak (1983) showed that regeneration of small, artificially-created lesions was more rapid in *A. palmata* than in *M. annularis*.

Montastraea annularis currently appears more vulnerable to diseases than *A. palmata*. Although almost 90% of the *A. palmata* colonies in studies around St. John had disease (primarily white pox) when observed in monthly surveys, many of these lesions healed. In contrast, growth of original colony tissue over white plague lesions has never been seen on *M. annularis* colonies.

It is possible that regeneration rates in *M. annularis* are slower than in the past. Also, the lesions associated with white plague are usually larger than those from white pox. White plague and Caribbean yellow band disease which disproportionately affect *M. annularis* and *M. faveolata* are especially virulent diseases (Bruckner and Bruckner 2006). Also, lesions (artificial or natural) surrounded by living tissue, such as those from white pox, may heal faster than those that border on bare or algal covered skeleton such as those from white plague.

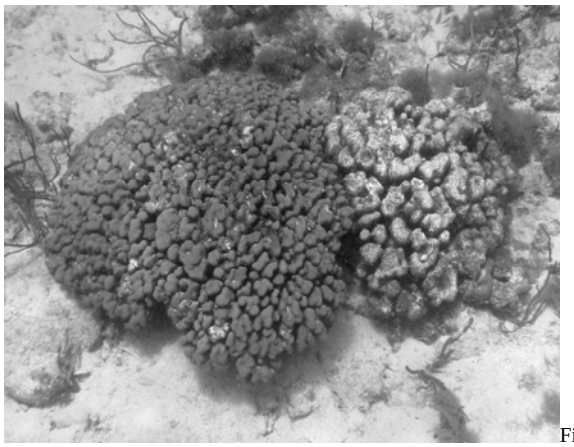

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Figure 1. Adjacent colonies of *M. annularis*, with the one on the left apparently healthy and the one on the right entirely dead.

Some colonies of both of these species seem to have more resistance to bleaching and disease than adjacent or nearby colonies (Fig. 1). Future research on different types of zooxanthellae and on the characteristics of different host coral genotypes may shed some light on this interesting phenomenon.

Evidence of recovery in the USVI and elsewhere

Acropora palmata is showing signs of limited recovery in the USVI, including in areas devastated by white band disease about 30 years ago (Gladfelter 1982, Rogers et al. 2003). Few people have followed *A. palmata* populations in precisely the same reef areas over time. Some signs of recovery have been noted at Buck Island Reef National Monument (Mayor et al. 2006), however these observations were before the major 2005 bleaching event that led to at least partial mortality of many colonies there (NPS, unpublished data). Our studies in St. John do not show clear evidence of an increase in number of colonies and in the overall amount of living coral tissue from 2003 through 2007 (Rogers et al. 2008a).

Recent observations indicate presence of new colonies of *A. palmata* in many areas (e.g., Macintyre and Toscano 2007, Zubillaga et al. 2008), but no clear signs of complete recovery back to the numbers (and overall colony condition) from two to three decades ago. One-time observations of the presence of this species are not irrefutable evidence of recovery. Macintyre et al. (2007) found no living *A. palmata* colonies on a bank-barrier reef off Barbados that was formerly dominated by this species.

Conclusions

The 2005 bleaching event and subsequent outbreak of disease caused the biggest decline ever recorded for

USVI coral reefs. No other stressors have caused so much loss, over such a large geographical area, in such a short time. Similar losses were observed in nearby Puerto Rico (Ballantine et al. 2008).

The fate of the coral reefs in the USVI is uncertain, largely because the fate of the major reef-building species, *Acropora palmata* and *Montastraea annularis*, is not known. Global climate change is expected to bring more intense hurricanes, warmer sea temperatures, more bleaching episodes, and ocean acidification, all of which are great concerns. How will these two species deal with these and other stressors in the future? Which of the stressors are amenable to local management and which must be managed on a global basis? What is the human component of global climate change? Extensive bleaching has not been seen on USVI reefs since 2005, but active white plague disease was seen in August 2008 at one of the St. John long-term study sites.

We are certainly not able to predict the future of the USVI seascape. We need to understand the links between human activities and diseases. Although *A. palmata* seems to have a better chance of recovery than *M. annularis*, based on its higher growth rate, greater potential for dispersal via fragmentation, and its greater resistance to bleaching and disease, there is no evidence of significant increases in the number and size of *A. palmata* colonies around the islands in the last five years. We also need to know more about water circulation patterns and connectivity between USVI reefs and upstream sources which have the potential to supply coral and fish larvae to replenish these degraded ecosystems.

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Black Band Disease upon the Reefs of Los Cayos Cochinos, Honduras

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Abstract. This study investigates Black Band Disease (BBD) incidence, distribution, and partial mortality effects upon scleractinian corals in the Marine National Monument, Cayos Cochinos (MNMCC). Searches for BBD used 80 m x 40 m plots at three different sites, chosen to represent a putative spectrum of site quality according to scleractinian coral species richness and percentage cover. The benthic community was analyzed using photo-quadrats, and BBD incidence figures were calculated. Colonies displaying BBD were tagged and photographed repeatedly to quantify the progression rate of disease. These same colonies were then re-photographed over the next two years to record colonization and succession data on the revealed skeletons. Incidence of BBD in MNMCC is low, with the impacted site showing only 0.1% of hosts infected. The healthiest site had an even lower incidence of BBD at 0.03%. The intermediate site had the highest incidence of BBD at 0.38%. BBD was found to follow linear and asymptotic patterns of progression rate. The Intermediate site, with the highest incidence was also found to have higher sediment flux than the other sites.

Key words: Black Band Disease, Honduras, Sediment, Coral Ecology.

Introduction

In the global degradation of coral reefs, coral diseases have been identified as one of the most important yet least understood causal factors (Harvell et al. 2004) For example it has been estimated that the disease, White Pox, has been responsible for reducing the population of the Elkhorn coral, *Acropora palmata*, by up to 87% in the Florida Keys during 1996 to 2002 (Sutherland et al. 2004). Black Band Disease (BBD) is unusual in being a microbial consortium upon the surface of a coral, rather than an internal infection (Carlton and Richardson 1995; Cooney et al. 2002; Frias-Lopez et al. 2002; Frias-Lopez et al. 2003). This microbial community is then responsible for creating a chemical environment that digests off and dissolves the coral tissue away from the skeleton (Richardson 1996). Unlike other coral diseases, BBD is often found in low incidences but persists within a reef for long periods, with the black band progressing over the surface of a coral at speeds of 3-10 mm per day (Antonius 1981; Edmunds 1991; Carlton and Richardson 1995). The distinctive patterning of healthy coral, black band and then recently killed white skeleton, makes BBD a distinctive infection on coral reefs. As such it is ideal for visual and ecological surveys, where laboratory analysis and microbiology techniques, such as Carbon Source Utilization Patterns, are not required to identify the pathogen. This facilitates the study of BBD in creating space on a reef for primary spatial competitors to corals, such as macroalgae and sponges. Sedimentation and elevated nutrients have been attributed as causal factors for increased disease prevalence on coral reefs (Bruckner et al. 1997; Kuta and Richardson 2002; Bruno et al. 2003; Nugues and Roberts 2003)

A study by the World Resources Institute, estimated that Honduran rivers provide 80% of all the sediment and over half of all the nutrients in the Mesoamerican Barrier Reef region (Burke and Sugg 2006). The Honduran Marine Protected Area, Marine National Monument Cayos Cochinos (MNMCC), is an archipelago consisting of two rocky islands and 13 smaller sandy cays. The archipelago is situated approximately 10 miles (16 km) off the coast of mainland Honduras and has been shown to be within reach of local river sediment plumes following the severe impacts of Hurricane Mitch in 1998 (Andrefouet et al. 2002). As such this area provides a unique opportunity to study BBD on reefs influenced by sedimentation and disturbance from local rivers (Paris and Cherubin 2008).

This investigation aimed to record BBD incidence at sites representing a spectrum of sediment impact and reef health. BBD progression rates and subsequent successional communities upon the corals were also recorded for inter-site comparison.

Material and Methods

Study site

Initially nine sites were surveyed with 100 m point transects to determine percentage cover of all benthic species. Additionally, scleractinian corals were identified to species level to form a gauge of reef health from percent cover and species richness (English et al. 1997; Hodgson 1999; Hill and Wilkinson 2004). Three sites were then selected out of the nine as putative healthy, impacted and intermediate sites (Fig.1.)

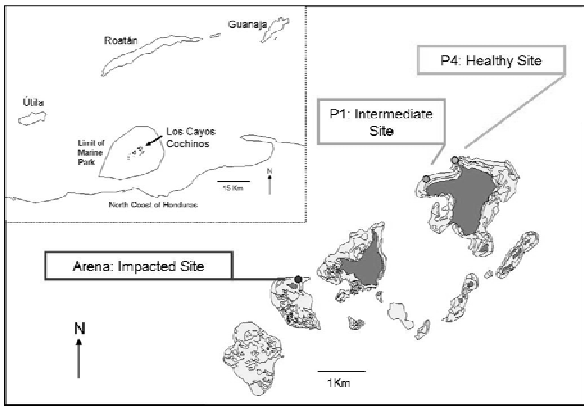

Figure 1: A map or the Marine National Monument Cayos Cochinos (MNMCC) and the three sample sites used in this investigation. Inset is a different scale map, showing the limits of the marine protected area and illustrating the relative close proximity of Los Cayos Cochinos to mainland Honduras. P1 is an abbreviation of Pelican 1 and likewise, P4 is Pelican 4.

At each site a 80 m x 40 m search area was created around permanent transect pins, which in turn had been used as a basis for the initial point transect surveys. The mid point of this 3200 m^2 search area was centered upon the reef crest at each site (6 m depth), thus encompassing both the reef slope and reef flat. 80 m x 40 m was chosen as it was the maximum search area that could be used within one dive, adhering to the local dive safety standards of 50 minutes per dive. Traditional belt transects and radial transects were tried (Kuta and Richardson 1996; Bruckner 2002), but did not cover a large enough area per dive to find incidences of Black band Disease (BDD). The ability to cover 3200 m^2 at each reef, on each dive, allowed daily comparisons of the three sites for new incidences of BDD, whilst monitoring existing infections. Between June and September 2005, colonies affected by Black Band Disease were identified and tagged using 3 mm diameter pins. These colonies were then photographed every day, and using Image Tool 3 software, progression rate and infection surface area were estimated. Colonies were then revisited in 2006 and 2007 for re-photographing and tracking of colony fate, in particular the colonization and succession of reef organisms on the post-BBD exposed skeleton. Other sources of mortality, such as bleaching, predation, burial by sediment or other diseases were also recorded with in the search area. Each 80 m x 40 m plot was then sub-sampled with photo quadrats to estimate the number of potential-host scleractinian colonies that could be infected by BBD according to the species list in Sutherland et al .(2004).

In order to compare site quality and any possible impacts from sedimentation, sediment traps were placed at each site, and suspended sediment sampled from the water column. Sediment traps were built from plastic tubing with a 9:1 optimum length to aperture internal diameter ratio (Bloesch and Burns 1980). This diameter was 7.4 cm and the trap length 67 cm. Baffles were fitted over the mouth to prevent large foreign objects falling into the traps, and to promote sediment capture and retention. Traps were placed on a 6 m isobath, in the center of each 80 m x 40 m plot. Traps were then recovered 7 days later, with the contents passed through pre-weighed Whatman Cellulose Filters (0.45µm) (Butler et al. 2008). Similarly, suspended sediment was determined by collecting one liter of water from near the traps during trap recovery, and then passed over similar pre-weighed filters. Comparison of filtrates allowed the calculation of sediment flux and inter-site comparison of sediment loads.

Results

Of the 14.2% hard coral cover found at the impacted site, Arena, approximately 50% (n=2133) of these corals were estimated to be potential host species for Black Band Disease (BBD). The overall incidence of BBD at this site was found to be 0.1% (n=2). At the healthy site, Pelican 4 (P4), hard coral cover was 15.4%, with around 30% (n=10664) of these corals estimated to be host species for BBD. BBD incidence at this site was the least at 0.03% (n=3). The intermediate site, Pelican 1(P1), had the highest incidence of BBD at 0.38% (n=15), but with lowest hard coral cover at only 5.5%, of which approximately 20% (n=4266) were estimated to be potential host species. The majority of infected corals (n=17 corals) were *Diploria strigosa*, with only 1 infection found upon a *Montastrea cavernosa* and 2 infections being upon *Diploria labyrinthiformis*. Infected corals were all found upon the reef flat at between 7 and 5 m depth.

Revisiting sites found dead portions of coral colonies were dominated by cyanophyta turfs within a month, then within 12 months succeeded by a an approximate 80% covering of *Dictyota* spp. macroalgae. However within 24 months post-BBD

mortality, the *Dictyota* coverage had been reduced, with *Porolithon* spp. covering the majority of the colony as clearly shown in Figure.2. Revision of the plots in 2006 found only 2 new incidences of BBD at Peli 4, 5 at Peli 1 and only 1 new incidence at Arena. In 2007 only 3 new incidences were found at Peli 1 and 1 incidence at Peli 4. No new incidences were found at Arena.

Analysis of BBD band progression rates found that not all infections progress at the same rate (Fig. 3.). Disease bands accelerate across a colony in linear fashions, but also progress in an asymptotic or sigmoid fashion, sometimes slowing down and dissipating altogether to leave patches of colonies alive in partial mortality events.

Sediment flux was found to be highest at Pelican 1, at 11.17 mg/m^2/d, with Arena having the lowest flux at 3.19 mg/m^2/d. Suspended sediment (Suspended Particulate Matter (SPM) was similar at all sites, ranging from 5.35 to 5.42 mg/L.

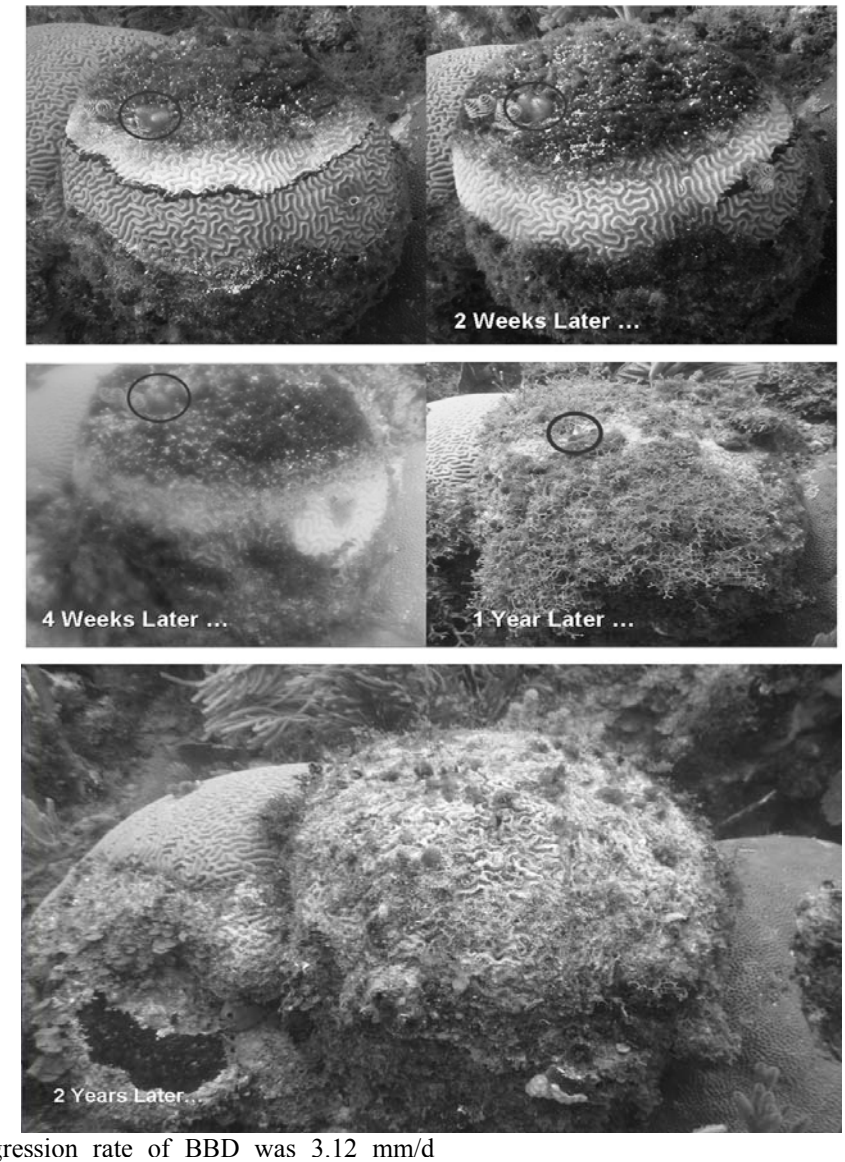

Figure 2: Succession of Black Band Disease and subsequent colonizers on a *Diploria strigosa* colony. Note rapid primary colonization by cyanophyta, then *Dictyota* spp., followed by succession to primarily *Porolithon pachydermum* cover. Circles highlight the same Christmas Tree worm cast as a point of reference

Mean progression rate of BBD was 3.12 mm/d (SE=2.65) at A and 1.45 mm/d

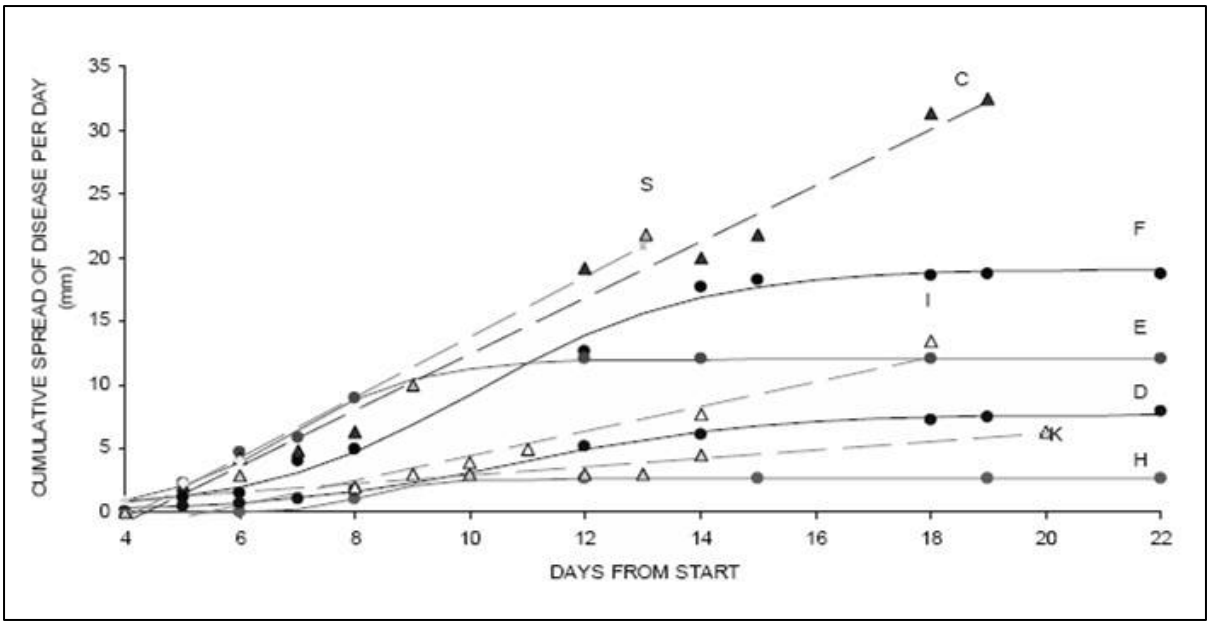

Figure 3: Cumulative spread of Black Band Disease upon 8 *Diploria* spp. colonies with linear response in triangles and dashed lines, and cessation of spread and asymptotic response after 7-14 days in circles and bold lines. Caps identify different colonies.

Discussion

Overall, Black Band Disease (BBD) incidence levels were found to be relatively low at the sample sites in Cayos Cochinos in 2005 (n=20), and continued to decrease in incidence through 2006 (n=8) to 2007 (n=4). This may be due to the period of increased water temperatures in 2005 (Rowlands et al. 2008). Increased temperatures could increase photosynthetic activity in the BBD consortium (Richardson and Kuta 2003) and in turn BBD virulence, or increased temperatures could abnormally stress corals, making them more susceptible to infection. The putative intermediate site in our study, Peli 1, had the highest incidence of BBD (0.38%). A possible explanation is that coral cover and potential host numbers are too low at Arena, the putative impacted site. In contrast the high coral cover and high species richness of the reef community at Peli 4 may be an indication that this putative healthy site is too 'healthy' for high BBD incidence. The sediment traps revealed the putative intermediate site, Peli 1, as having the highest sediment flux (11.17 mg/m^2/d). This higher sediment load than the other sites maybe directly causing BBD, acting as a substrate for cyanophyta mat formation or acting as a source of stress for corals, leaving colonies more susceptible to infection Dissolved nutrients have been found at only trace levels at all sites (Shrives 2007), but a future study could use Nitrogen and Carbon Isotope data or coral cores to better understand the nutrient regime at these sites.

BBD was found to play a part in patch dynamics upon the reef flats of Cayos Cochinos. The progression rate data (Fig.3.) illustrates that not all mortality from BBD resulted in entire colony death, leaving some patches of coral alive. Not all corals suffered from terminal *Dictyota* spp. domination, and often there was a succession to *Porolithon* spp. cover, suggesting that there is either still a reasonably good population of herbivores nearby, or that *Dictyota* spp. are dying back during the winter months. BBD was also observed to be an indirect source of mortality for the Christmas Tree worm, *Spirobranchus giganteus*, often abundant on the *Diploria* spp. colonies photographed and tagged within this study. Post BBD colonies supporting *Spirobranchus giganteus* became obscured by the growth of *Dictyota* spp. and died. In turn, the vacated *S. giganteus* tubes were observed and photographed as a new source of habitat and refuge for Blenniidae.

In conclusion, although found at relatively low incidence levels in Cayos Cochinos, Black Band Disease was found to contribute to the creation of primary space upon these reefs, playing a role in patch dynamics. Incidence levels and progression rates did not correlate as expected with the initial site classification for reef health using hard coral cover and species richness. BBD was also observed to have indirect effects upon the ecology of other fauna around the reef. Although sediment flux was found to

be highest at the study site with highest incidence of BBD, increased site replication and further studies are required to attribute any direct causal factors to disease outbreaks upon the reefs of the Honduran Marine National Monument, Cayos Cochinos.

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Coral Yellow Band Disease; current status in the Caribbean, and links to new Indo-Pacific outbreaks

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Abstract
Yellow band disease (YBD) has had severe impacts on major reef-building corals throughout the Caribbean. Recent data from Bonaire indicates that this disease remains in an epidemic phase showing similar trends compared to the late 1990s. Ten meter belt transects taken at varied depths of *Montastraea* spp. indicate high indices of yellow band lesions. At 5m depth, yellow band rings and lesions were positively identified on an average of 9.4 (87%) colonies per transect while an average of 1.2 (13%) colonies in this depth range appeared healthy. At 10m depth an average of 1.0 (12%) healthy colonies and 8.3 (88%) colonies affected by YBD per 10m transect were counted. At 15m there were an average of 0.7 (17%) healthy colonies and 2.6 (83%) colonies affected by YBD per 10m transect. These studies coincide with recent severe outbreaks in the Indo-Pacific where similar lesions were found on *Diploastrea* spp., *Herpolitha* spp.and *Fungia* spp. Inoculation experiments of Indo-Pacific bacterial isolates under ambient temperatures cause the induction of YB lesions onto *Montastraea* spp. *in vitro*. YBD continues to be in an infectious stage in its original Caribbean hot spots and appears to be spreading in Pacific coral genera.

Key Words: Yellow band, *Montastraea* spp., zooxanthellae, symbiosis

Introduction

The severity of Yellow band/blotch disease (YBD) affecting reef-building Scleractinian corals in the Caribbean (Goreau et al. 1998, Santavy et al. 1999, Cervino et al. 2000, Weil et al. 2006), and Indo-Pacific has been widely documented and has been linked to *Vibrio* pathogens that are genetically related to *V. alginolyticus* and *V. harveii (*Cervino et al. 2004*)*. Yellow band disease targets *Montastraea* spp. throughout the Caribbean and *Fungia* spp., *Herpolitha* spp., and *Diploastraea heliopora* in numerous locations in the Indo-Pacific (Cervino et al. 2008). This is not the first time that *Vibrios* have been implicated in a coral disease; Kushmaro et al. 1996, suggested that vibrionic bacterial bleaching is caused by a temperature-influenced infection by *Vibrio shiloi* (Ben-Haim and Rosenberg 2002). *Vibrio corallilyticus* was also shown to cause bleaching and tissue lysis in the Indo-Pacific coral *Pocillopora damicornis* (Ben-Haim et al. 2003)**.** YBD is having an adverse affect on abundances of these species as rates of infection have reached epidemic proportions.

Research from 1997 to 1998 in Bonaire showed that 91% of *Montastraea* spp. corals at ten research sites were affected by YBD (Cervino et al. 2000). Ten years later, belt transects counted in Bonaire from 2007 to 2008 at the research site Karpata reveal that 86% of *Montastraea* spp. corals are currently affected. Observations of YBD at numerous other sites in Bonaire and Klein Bonaire are consistent with the transect analysis conducted at Karpata.

Yellow band/blotch disease begins as a small lesion (or blotch) about 1-2 cm in diameter. Polyps in the lesion area acquire a somewhat irregular and swollen appearance and pigmentation is lost, giving the affected area a lighter aspect than surrounding healthy tissue. The disease spreads at a rate of about 0.5 cm to 1 cm per month. Gradually, the lesion becomes a yellow ring (or band) of diseased tissue bordered by necrotic tissue often overgrown with algae within and healthy tissue surrounding it. As the diseased tissue advances the ring/band and the dead tissue and algae overgrowth area expands (Fig. 5). The virulence of YBD is greatly enhanced by warmer seawater temperatures (Cervino et

al. 2004) which in the context of global climate change and El Niño events could mean that coral colonies will disappear quicker than they can recover or reproduce (Colwell, R. 1996, Goreau et al. 1998, Harvell et al. 1999, Van Veghel and Bak 1994).

Recent outbreaks of YBD off the fringing reefs of the west coast of Barbados (Brathwaite, pers. comm), together with the list of documented areas where YBD has already been identified (Fig. 1) demonstrate that the disease continues to spread throughout the Caribbean.

Figure 1: YBD has been documented throughout the Caribbean in; Antigua, Bahamas, Barbados, Belize, Bermuda, BVI, Cayman Islands, Colombia, Cuba, Dominican Republic, Florida, Grenada, Jamaica, Mexico, Netherlands Antilles, Panama, Puerto Rico, Roatan, San Salvador, St. Kitts, St. Maarten, St. Vincent and the Grenadines, Turks & Caicos, USVI, Venezuela

Recent field research in Indonesia, the Philippines and Thailand and subsequent laboratory research show that similar lesions on Indo-Pacific coral species are caused by the same pathogens that cause YBD in Caribbean coral species (Cervino et al. 2008). More research is needed in this vast geographical region to determine how widespread vibrio-induced YBD infections currently are, in order to understand the long-term impact on Indo-Pacific coral reefs.

Materials and methods

Prevalence of Yellow band/blotch disease was determined by belt transect counts using SCUBA. Surveys were conducted in August of 2007 and January of 2008. An average of ten transects each (100 linear meters) at depths of five meters, ten meters, and fifteen meters were counted. North and south directions from the site's buoy were covered to avoid overlap of transects done from August 2007 to January 2008.

Bonaire is home to a very large population of *Montastraea* spp. corals and boasts one of the most successful Marine Protected Areas (MPAs) in the Caribbean. Due to these attributes, Bonaire presented a unique opportunity to gauge the status of the region's *Montastraea* spp. health, by studying this area well protected from overfishing, boat anchors, rampant coastal overdevelopment and other stressors that plague the rest of the Caribbean. The study site, Karpata, is a very popular dive site on the northwest coast of Bonaire and has a particularly abundant and densely packed population of *Montastraea* spp. colonies (Bak et al 2005).

Temperatures in August of 2007 ranged from an average of 29° Centigrade at five and ten meters of depth to an average of 28° Centigrade at fifteen meters depth. In January of 2008 temperatures averaged 26° Centigrade at all depths. Only minor bleaching was observed.

An underwater reel with ten meters of measured nylon line was stretched horizontally along the reef and held in place by two divers constantly monitoring depth and tension of the transect line. The survey was conducted along the transect line and all colonies that crossed the line were counted. It should be noted that the underwater topography at 10 and 15 meters depth is a near vertical wall, therefore colonies that fell into the line of sight *behind* the transect line on the wall were counted while at five meters depth the transect line was stretched above the flat reef and all colonies that lie in line of sight *below* the transect line were counted. A series of photos was taken along transects at all depths using an underwater Sony W5 digital camera in August of 2007.

Montastraea spp. colonies with visible signs of YBD were counted as infected. Colonies devoid of visible signs of lesions/blotches or rings/bands and those that showed signs of disease that could not be positively attributed to YBD were counted as healthy. "Healthy" in the context of this paper does not mean that coral colonies were free of other diseases, it means only that Yellow band/blotch disease was not present on that particular colony.

Results and Observations

A survey of the incidence of Yellow band/blotch disease at Karpata was conducted to determine the status of *Montastraea* spp. ten years after the previous survey. Overall, the indices of YBD were very high (86%) in 2007/2008, similar to the findings in 1998 (91%) (Cervino et al. 2000).

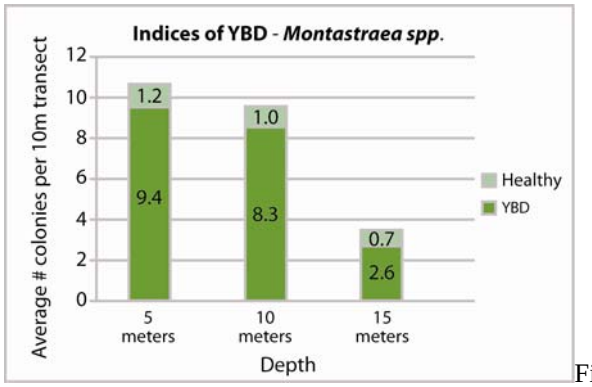

Figure 2: YBD transect results 2007 & 2008 in Bonaire showed high indices of disease as compared with healthy corals.

At five meters depth, an average of 9.4 *Montastraea* spp. colonies per ten-meter belt transect were infected with YBD, as opposed to an average of 1.2 healthy colonies. At the top of the reef, in the five-meter range, many colonies are lobate *Montastraea annularis* or relatively small colonies of *Montastraea faveolata* whose morphology may explain why smaller blotches were the most observed sign of disease at this depth. There remains a relative abundance of live coral cover at this depth though the majority (87%) of colonies show signs of infection.

At ten meters, the sloping wall has a large number of densely packed, mountainous *Montastraea faveolata* colonies. These colonies are often larger than the colonies counted at five meters and the surface area of the *M. faveolata* is by and large more continuous than that of *M. annularis*, which is abundant at shallower depths. An average of 8.3 *Montastraea* spp. colonies per ten-meter belt transect showed signs of YBD while 1.0 colonies appeared to be healthy. Relative abundance of live *Montastraea* spp. colonies is lower at ten meters than at five meters however the incidence of corals infected with YBD was slightly higher at eighty-eight percent (88%).

The reef at fifteen meters is topographically similar to the reef at ten meters. An average 2.6 *Montastraea* spp. colonies per 10-meter belt transect were found to be infected with YBD and an average of 0.7 colonies were exempt from signs of the disease. The number of colonies counted at this depth was noticeably lower since most *Montastraea* spp. colonies at this depth were dead or nearly dead (more than 75% loss of live coral tissue) and were overgrown with algae, sponges, tunicates and recruits of other coral species. Many of these colonies were at such advanced stages of tissue loss that it was difficult to diagnose the cause of death. Overall, eighty-three percent (83%) of *Montastraea* spp. colonies that remain at this depth were infected with YBD.

Similarly, at six field sites in the Wakatobi Island chain, Indonesia 15 m x 1 m belt transect counts were conducted which found 34 (19%) *D. heliopora* colonies infected with YBD and 531 (42%) *Fungia* spp. colonies infected with YBD. These numbers reflect the dominance of *Fungia* spp. at the Wakatobi reefs and show an analogous trend to the outbreak of YBD on the primary reef-building *Montastraea* spp. colonies in Bonaire. It would appear, then, that an abundance of a particular susceptible species yields high indices of YBD infection and its spread may be density dependent.

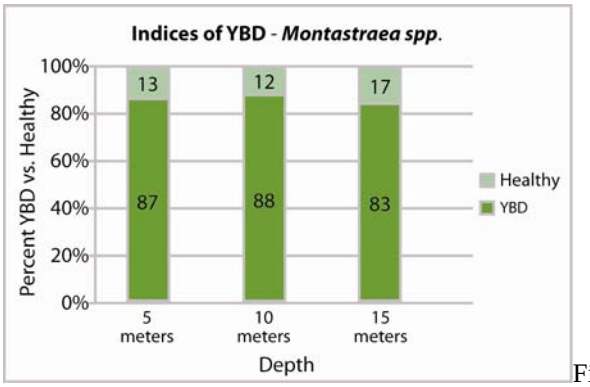

Figure 3: Percentage of YBD infected *Montastraea* spp. colonies in Bonaire - transect results 2007 & 2008

Discussion

According to recent reports from scientists and divers, *Vibrio* induced Yellow band lesions throughout the Caribbean have not diminished. Nor have YBD infected colonies in Bonaire recovered since they were reported in1997. *In situ* studies and recent new outbreaks indicate that this epidemic has lasted over a decade in the Caribbean and Florida.

YBD is caused by a consortium of four novel *Vibrio* spp. together with *Vibrio alginolyticus*, a well-known shellfish pathogen (Cervino *et. al*. 2008). YBD is a disease of the symbiotic algae and not of the host coral as the microbial consortium attacks the zooxanthellae *in situ* within the gastroderm causing lysing of zooxanthellae (Cervino et al. 2004). Vacuolization and fragmentation occur and severe impairment of the thylakoid membranes cause loss of chlorophylls a and c_2, which impairs photosynthetic functionality. (Cervino et al., in prep).

Were it not for the important benefits of association with their symbiotic algae, coral hosts would be unable to lay down calcium carbonate skeleton at the same rate as they have done for centuries. Benefits of photosynthesis and fixation of carbon provided by the zooxanthellae are necessary for the growth of the coral host (Muscatine and Cernichiari 1969, T.F. Goreau et al. 1979, Muller-Parker and D'Elia 1997). Corals that are infected with YBD and other diseases experience slower

growth rates, and due to a great net loss of biomass, coral colonies experience a reduction in ability to reproduce (Antonius, A. 1977 1981, Peters 1984, Kojis and Quinn 1985, Szmant 1991, Goreau et al. 1998, Porter et al. 2001).

The exact mechanism by which the *Vibrio* consortium actually causes disease is still unknown. However, we hypothesize that the mode of adhesion is similar to what Banin et al. *(2001)* showed when *Vibrio shiloi* biosynthesized and secreted an extracellular peptide - toxin P - that impaired and inhibited photosynthesis of symbiotic zooxanthellae. The first physical barrier to infection for most corals is the mucopolysaccharide layer, which in healthy coral colonies is home to a plethora of coral-associated microbes that play an important role in antibacterial activity (Hayes and Goreau 1998). Pathogenic stress, or environmental stress, such as a thermal bleaching event, can alter the microbial community commonly associated with a particular species (Frias-Lopez et al. 2002, Bourne et al. 2007). It is possible that opportunistic microbes – in this case, the YBD *Vibrio* consortium - repopulate the mucus layer after such events, causing disease (Knowlton and Rohwer 2003, Ritchie, K.B. 2006, Gil-Agudelo, D.L. et al. 2007, Rosenberg et al. 2007).

These factors have great implications for the health and abundance of the major Caribbean reef-building *Montastraea* spp. colonies whose numbers are in decline throughout the region.

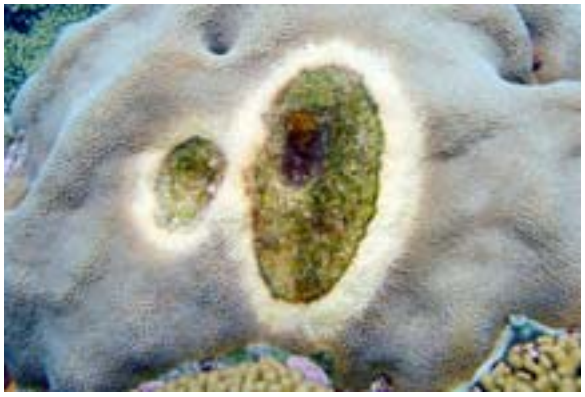

Figure 5 – Montastraea annularis with classic signs of YBD infection. Photo was taken in Bonaire, August 2007. Photo credit: Alessandro Donà.

Yellow band/blotch disease in the Indo-Pacific

YBD is currently also affecting reef-building corals in the Indo-Pacific. Field studies and laboratory experiments by Cervino et al. have shown that similar pathogens are causing similar symptoms of disease in *Fungia* spp., *Diploastraea heliopora*, and *Herpolitha* spp. colonies.

Inoculation experiments, conducted at the Mote Marine Laboratory in Summerland Key, Florida in 2007, of *Montastraea faveolata* fragments with the *Vibrio* spp. isolated from diseased Indo-Pacific corals in aquaria resulted in YBD infection (Fig. 4). Interestingly, inoculation of the *M. faveolata* fragments from the 26° and 28° C tanks resulted in infection while identically inoculated fragments in the 30° and 32° C tanks did not result in infection.

Figure 4 – M. faveolata fragments from 26° C tank, inoculated with Indo-Pacific consortium, resulted in YBD lesions.

It is important to note that Yellow band disease infections were not found to be more prevalent with warmer seawater temperature. However, when YBD-infected corals from ambient temperature tanks were moved to warmer temperatures the disease became more virulent. In other words, corals are apparently susceptible to YBD infection when seawater temperatures are normal, and the disease is more likely to spread during warmer periods.

Conclusion

There is still much to understand regarding the mechanism of host-alga transmission that leads to infection in YBD. That need is more compelling than ever in light of the recent Indo-Pacific outbreaks and the connection between infectivity at normal seawater temperatures and virulence with rising seawater temperatures. As Cervino et al. 2004 2008, have shown, YBD is no longer only a disease of Caribbean coral species, but it is now having a large impact on the reef-building corals of the Indo-Pacific. Further investigation should include laboratory analysis of the zooxanthellar clade subtypes that are affected and YBD's possible connection to aquaculture pathogens. The negative implications of a widespread infectious disease such as YBD are numerous. Besides disease, corals must deal with myriad debilitating stressors – both anthropogenic and non - such as predation, bleaching events, pollution, sedimentation and overfishing. The environment is

quickly changing; the sum of all these ills and the accelerated timescale might be more than corals can adapt to. A strong working knowledge of the problem is essential for effective coral reef management and efforts to mitigate the damage to coral reefs around the world.

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Role of the coral surface microbiota in disease: an *in situ* test using the *Gorgonia-Aspergillus* pathosystem

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Abstract. The surface mucopolysaccharide layer (SML) is an energy rich environment that supports host-specific microbial communities. Studies have shown that the coral surface microbiota shift, in both richness and abundance, correlated with environmental perturbations and pathogens. Thus, surface microbiota may play a mutualistic role in the health of the coral host, analogous to the role of human gut microbiota. Indeed, we hypothesize that the coral surface microbiota play an integral role in disease resistance and that perturbation of the microbiota increases disease susceptibility. Here, we report on *in situ* experiments to test whether the structure of the surface microbiota of the Caribbean sea fan, *Gorgonia ventalina,* responds to perturbations in the local environment. Specifically, we tested the effects of light reduction, nutrient enrichment, antibiotic wash, and pathogen (*Aspergillus sydowii*) exposure on the microbiota as characterized using DGGE. Results indicate that the structure of the surface microbiota changes in response to perturbation and that some bacterial strains were present in untreated control corals and remained throughout all treatments. This provides experimental evidence that the surface microbiota are a link between the coral, their environment, and potentially their health. Yet the response to the perturbations appears to be complex, and most likely are the result of primary and secondary interactions between the environment, microbiota, mucus, and coral host.

Key words: Surface mucopolysaccharide layer, Microbiota, Pathogen, Perturbation, DGGE

Introduction

The correlation of incidence of coral disease with elevated seawater temperatures (Hoegh-Guldberg 2004), increased pollutants (Kim and Harvell 2002; Kaczmarsky et al. 2005), nutrient enhancement (Bruno et al. 2003), and light intensity (Richardson and Kuta 2003) suggest that environmental conditions play a role in pathogenesis. At present, the mechanisms connecting environmental factors and disease are not well understood. However, recent studies have pointed to microbial associates of corals as a possible link between environment and disease (Kaczmarsky 2006; Reshef et al. 2006).

Coral microbial associates reside primarily within or upon a surface mucopolysaccharide layer (SML) secreted by the coral host (Rohwer et al. 2002) to protect against UV light, desiccation, and to cleanse them of sediments. The SML consists of polysaccharides, proteins, lipids, and fatty acids (Meikle et al. 1988) and represents an important nutrient source in reef ecosystems (Wild et al. 2004). Microorganisms, take advantage of this with the SML supporting species-specific (Rohwer et al. 2002) and abundant microbial communities (Ritchie, Smith 1995; Brown and Bythell 2005).

However, given the paucity of information about the role of SML microbial communities of corals, hereafter referred to as surface microbiota, the vertebrate system provides a useful model for gaining insights and developing testable hypotheses. For instance, vertebrate surface microbiota act as an obstacle to pathogen colonization (Fredricks 2001; Bojar and Holland 2002). Perturbation of the microbiota from antibiotics, for example, may facilitate proliferation of less abundant and potentially pathogenic strains of bacteria, or allow non-resident microbes to colonize (Lysenko et al. 2005). This function of resistance by the microbial community is called 'colonization resistance' and is an important part of disease resistance (van der Waaij 1989).

In corals, like vertebrates, some of the surface microbes may confer disease resistance. Indeed, Reshef et al. (2006) suggest that the microbiota of corals are 'probiotic' in that the surface microbiota adapt and facilitate resistance against pathogens. Here, we hypothesize that the surface microbial communities change in response to exposure to a pathogen and environmental perturbation. If the SML microbial communities do change, then this provides evidence that surface microbiota may help mediate coral health. Shifts would indicate a stressed system

and could represent increased disease susceptibility. Consistent with this hypothesis, several studies have reported that surface microbiota of diseased corals are different from healthy ones when compared quantitatively and qualitatively but have not included environmental effects. For example, different surface microbial communities were documented on diseased areas of *Montastraea annularis* corals than on healthy (Frias-Lopez et al. 2002; Pantos and Bythell 2006), and a more diverse microbial community has been detected in diseased relative to healthy corals (Bythell et al. 2002; Pantos et al. 2003). Changes to the microbiota of corals were also found during times of environmental stress, such as during thermal bleaching events (McGrath and Smith 1999). Also, differences in the microbiota were detected on corals depending on coastal proximity and depth (Klaus et al. 2005) as well as across seasons (Guppy and Bythell 2006).

While these correlative studies suggest that the microbiota respond to environmental change, to date, no direct experimental evidence has been provided. The goal of this research is two-fold: first to document experimental perturbation of the coral surface microbiota, and second, to provide support to what we refer to as the "Coral Microbiota Disease Hypothesis." This hypothesis posits that the surface microbiota play an integral role in mediating disease susceptibility and that any perturbations to the structure of the microbiota increase disease susceptibility. Using the *Gorgonia-Aspergillus* pathosystem, coral fragments were subjected to treatments of antibiotics, light disruption, and nutrient enhancement *in situ* to document changes in coral surface microbiota.

Material and Methods

Study site. All of the field collections and experiments were carried out at Pickles Reef (24°59.079' N, 80°24.978' W), located 14.5 km off Key Largo in the Upper Florida Keys during August 2006.

Experimental Design: Three fragments (~16 cm^2) were cut from the edge of *Gorgonia ventalina* sea fans and haphazardly assigned to a treatment, a control, and initial sample. The initial sample was sealed in a sterile plastic bag and transported to the lab in a cooler filled with seawater from the collection site for processing. The two additional fragments were fastened onto a PVC rack that was anchored to the reef substrate for 10 days. Using this design with the coral fragments (same genotype exposed to different treatments), the following three experiments were carried out.

Antibiotic Treatment. The coral fragments from two apparently healthy colonies were treated with an antimicrobial wash (50 μgml^{-1} gentamycin, 100 μgml^{-1} rifampicin, and 100 μgml^{-1} streptomycin in sterile seawater as per Buyer et al. (2001)). Half of the fragments were inoculated via attachment of a pathogen strip consisting of a sterile piece of gauze (1 x 2 cm) embedded in a thin layer of agar overgrown with the fungal pathogen *Aspergillus sydowii*.

Light Disruption. Apparently healthy coral fragments from three colonies were anchored to PVC frames on the reef substrate and were covered by shade cloth laid over a clear sheet of plexiglass (to provide structural support for the shade cloth). As a control, fragments were put under the plexiglass only.

Nutrient enhancement. Increased nutrients were administered to coral fragments taken from three apparently healthy or three actively diseased parent colonies and fastened to the PVC frames. This was done by placing time-release fertilizer (Osmocote Flower and Vegetable Plant Food, N-P-K: 14-14-14, Marysville) in "nutrient pillows" per Bruno et al (2003) that were secured next to each anchored fragment. Control pillows were filled with rinsed pebbles.

Mucus Collection and DNA Extraction: All coral fragments were collected after 10 days and transported to the lab. Mucus was collected from each fragment by vortexing for 1 min in 40 ml of sterile seawater. After vortexing, the coral fragment was removed and the remaining slurry was stored at -80°C for three months until molecular analysis. Mucus and seawater samples were thawed and centrifuged three times (5 min at 10,000x g) and the DNA in the mucus concentrate was extracted using protocol outlined in the Power Soil DNA Isolation kit (Mo Bio, California) and eluted to a final volume of 1000 µl. DNA concentration was determined using a spectrophotometer set at 260 nm.

PCR Amplification: A nested PCR procedure was used to amplify bacterial 16S rDNA using primer sets previously described to analyze coral bacteria (Pantos et al. 2003). The first round of PCR used primers pA (8F) and pH'(1542R) with a minimum DNA concentration of 10 ng/µl. A touch-down PCR cycle was used following protocol from (Cooney et al. 2002). Products were verified by agarose gel electrophoresis and 5µl used as template for the second round of amplification using the primers Pc (341F) with a GC rich clamp incorporated to the 5' end, and pE'(928R). The nested products were purified and concentrated to 30 µl using a QIAquick purification kit (Qiagen, Valencia).

DGGE Analysis: Denaturant gradient gel electrophoresis was conducted using The D-Code universal mutation detection system (Bio-Rad, California). The nested and purified products were resolved on 6% (w/v) bis-acrylamide gels across a 25 to 50% denaturant gradient to select for fragment

number and sizes appropriate for analysis. 10uL of each nested product was loaded into each lane and run for 12 h at 90 V at 60° C. Gels were stained with Sybr Gold (Sigma, California) for 20 min and visualized with a UV transilluminator. Gel images were captured using Quantity One D-Code imaging software (Bio-Rad, California).

Statistical and OTU analysis: Bacterial operational taxonomic units (OTUs) seen as bands were defined by band-matching analysis as part of the Quantity One D-Code imaging software (Bio-Rad, California). The inclusion of uncertain bands was based on sensitivity of > 1%, such that a band was recognized relative to background pixels color intensity. A 1kb mass ladder (Fisher Scientific, Pennsylvania), manually defined in the software was used in a marker lane for between-gel comparisons. Mean OTUs and treatment effects were analyzed across all gels by 1-way ANOVA and 2-tailed T-tests (JMP). Multivariate cluster analysis (Mean Hierarchical algorithm, JMP, Version 5) were done by comparing samples to composites of OTUs using presence/absence of bands, to determine similarity in DGGE profiles.

Results

Out of all the samples there was a maximum of 22 OTUs, with presence or absence of at least 1 to 8 unique OTUs that correlated with coral treatment. Arrows labeled 5 and 6 indicate unique bands present in some samples but absent in others from the DGGE gel. There were also OTUs common across all treatments, including seawater and first round amplification control samples at arrows 1-4, and 10 (Fig.1). The presence of bands in the seawater, filtered water, and first round amplification (C1) indicate microbes similar to all SML samples and seawater amplified during the first round of nesting. Yet absence of bands in lane labeled C2 indicates that there was no contamination after two rounds of amplification.

The mean richness, determined as total OTUs per mucus sample, across all treatments in all gels was 11 (SE=2.66). The minimum OTU richness was 4 with maximum of 22 and the mode was 8. Overall, OTU richness showed no significant difference among samples of the same environmental perturbation treatments (1-way ANOVA, Antibiotic: F=0.05, P=0.99; Light: F= 0.148, P=0.87; Nutrient: F= 0.60, P=0.56; 2nd & 3rd gels not shown; Figs. 2A, 3A, 4A).

The DGGE data were analyzed using a cluster analysis based on OTU presence/absence to reveal similarities among communities of SML microbiota (Figs. 2B, 3B, 4B & 5).

Antibiotic Treatments. Cluster analysis revealed OTU profiles from the initial healthy sample and the combination treatment of pathogen and antibiotic together, were more similar to each other than to the control (Fig.2B).

Light Disruption. Cluster analysis revealed profiles of the healthy and shaded samples were more similar to each other than to the control (Fig.3B). The initial samples from like colonies were more similar to the control than the shaded.

Nutrient Enhancement. Cluster analysis indicated OTU mucus profiles from initial disease, pathogen only, and the nutrient enhancement, shared 8 OTUs from a composite total of 20. Together these profiles were more similar to the control, than the initial healthy. The sample that was both diseased and nutrient enhanced was more similar to the initially healthy than the initially diseased. The two most dissimilar profiles were the initially healthy and initially diseased, with difference of 10 OTUs, yet both when subjected to increase in nutrients became more similar (Fig.4B).

All treatments. Overall, cluster analysis revealed OTU profiles were more similar based on original sea fan colony than on treatment, although more similar profiles were obtained from like treatments than not (Fig.5).

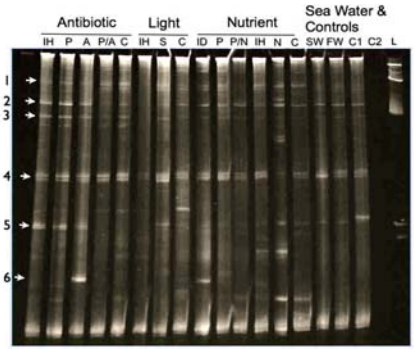

Figure 1: DGGE image depicting 16S rDNA profiles. Bands of operational taxonomic units (OTUs) represent possible unique strains of bacteria. Numbered arrows indicate OTU sites unique to some treatments (5 & 6), and those found across all treatments (1-4). Lanes are labeled by treatment given to coral fragments: IH=initial healthy, P=pathogen, A=antibiotic, C=control, S=shaded, ID=initial diseased, N=nutrients, FW= filtered water.

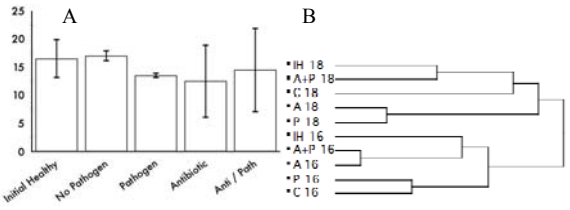

Figure 2: Mean OTU Richness (A) and Similarity of Molecular Profiles (B) from Antibiotic Treatments. Richness (± SE) is from all gels. Clusters are labeled by treatment given to coral fragments: IH=initial healthy, P=pathogen, A=antibiotic, C=control, S=shaded, ID=initial diseased, N=nutrients. Numbers indicate the original fragment colony.

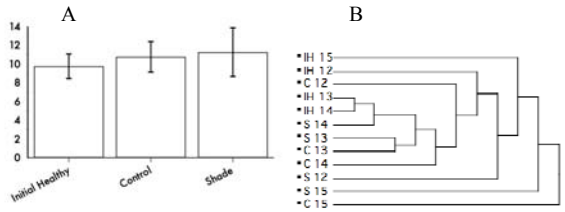

Figure 3: Mean OTU Richness (A) and Similarity of Molecular Profiles (B) from Light Disruption Treatments. Richness (± SE) is from all gels. Clusters labeled by treatment given to coral fragments: IH=initial healthy, P=pathogen, A=antibiotic, C=control, S=shaded, ID=initial diseased, N=nutrients. Numbers indicate the original fragment colony.

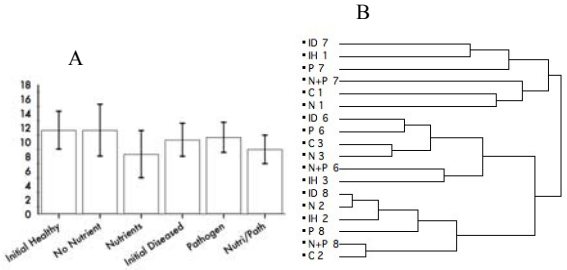

Figure 4: Mean Bacterial Richness (A) and Similarity of Molecular Profiles (B) from Nutrient Enhancement Treatments. Richness (± SE) is from all gels. Clusters labeled by treatment given to coral fragments: IH=initial healthy, P=pathogen, A=antibiotic, C=control, S=shaded, ID=initial diseased, N=nutrients. Numbers indicate the original fragment colony.

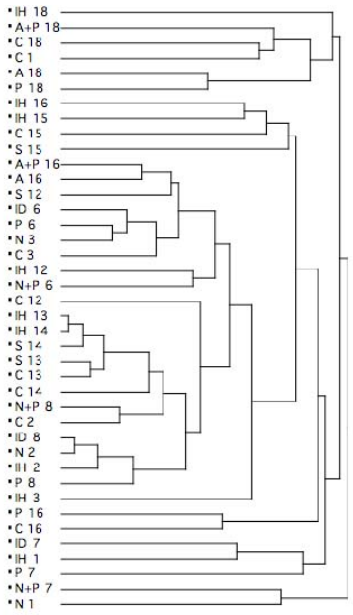

Figure 5: Similarity of Molecular Profiles across all treatments. Clusters labeled by treatment given to coral fragments: IH=initial healthy, P=pathogen, A=antibiotic, C=control, S=shaded, ID=initial diseased, N=nutrients. Numbers indicate the original fragment colony.

Discussion

Variations in molecular profiles of the coral SML microbiota were detected when sea fan fragments were infected by a pathogen, treated with antibiotics, subjected to reduced light levels, and exposed to elevated nutrient levels *in situ*. Some OTUs, potential bacterial strains, were present in control corals and remained throughout all treatments. This finding is consistent with the Coral Microbiota Disease hypothesis that posits that surface microbiota play an integral role in mediating disease susceptibility.

The shifts in the microbes demonstrated by this study may not have been statistically significant because of the DGGE gradient used. It is possible that more OTUs could have been detected because there appeared to be high concentrations of DNA the top and bottom of the gel (Fig. 1). Another consideration is that PCR bias may have caused preferential amplification of sequences that resulted in more similar OTU profiles. PCR artifacts have been shown to effect results of 16S rDNA diversity analysis (Acinas et al. 2005).

The microbes may in fact be responding to a change in mucus quantity or quality and not directly to the treatments. The microbial communities living in the SML are thought to rely on exudates from the coral-zooxanthellae association (Ducklow and Mitchell 1979; Gil-Agudelo et al. 2006). It would follow that alterations in the microbial community would occur if the coral-zooxanthellae relationship were altered. A change in nutrient and or light availability may alter the coral-zooxanthellae symbiosis and in turn affect the nature of the SML qualitatively (i.e., composition) or quantitatively. For instance, studies have also shown that copious mucus production is often the first visible sign of a generalized response by corals to environmental stress (Peters 1984).

Not to be disregarded are the potential roles of innate host response and microbial interference. Kim et al. (2000) demonstrated that the sea fan *G. ventalina* is capable of a chemically mediated inducible response against the pathogen *A. sydowii.* It may take considerable amounts of energy for the corals to produce these defensive compounds, which come at the expense of secreting the protective mucus layer (Edmunds and Davies 1989). It would follow then, that alterations in the microbial communities, which utilize the SML, would result. Alternately, diseased corals may reduce the production of these compounds so that they can maintain "normal" mucus production.

It was suggested that *G. ventalina* may respond to *A. sydowii* with a non-specific antimicrobial product regardless of whether it is fungal or bacterial (Alker et

al. 2004). The inhibitory compounds could affect non-targeted microbes. Also, some SML microbiota themselves produce antimicrobial compounds (Castillo et al. 2001; Ritchie 2006).

In the present study, host factors did appear more important to microbial diversity than direct perturbation. Particularly in the cluster analysis from all treatments that show the sea fan of origin was a better predictor of microbial similarity than the pathogen or perturbation it was exposed to. Yet, due to a lack of knowledge about microbial interactions, innate host response, SML antimicrobial products, and environmental effects, it is hard to know if the microbial shifts noted herein were affected by any or all of these factors.

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PCR-based Assay for Detection of Four Coral Pathogens

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Abstract. Several microorganisms have been identified as pathogenic agents responsible for various outbreaks of coral disease. Little has been learned about the exclusivity of a pathogen to given disease signs. Most pathogens have only been implicated within a subset of corals, leaving gaps in our knowledge of the host range and geographic extent of a given pathogen. PCR-based assays provide a rapid and inexpensive route for detection of pathogens. Pathogen-specific 16S rDNA primer sets were designed to target four identified coral pathogens: *Aurantimonas coralicida, Serratia marcescens, Vibrio shilonii,* and *Vibrio coralliilyticus*. Assays detected the presence of targets at concentrations of less than one cell per microliter. The assay was applied to 142 coral samples from the Florida Keys, Puerto Rico, and U.S. Virgin Islands as an *in situ* specificity test. Assays displayed a high-level of specificity, seemingly limited only by the resolution of the 16S rDNA.

Key words: coral disease, pathogen, bacteria, detection assay, PCR.

Introduction

The study of coral microbiology has been largely fueled by investigations of disease. These studies have been aimed at identification of specific pathogenic agents, followed by the classical method for confirmation of disease causation by fulfillment of Koch's Postulates (Koch 1882). In this manner, numerous putative pathogenic agents have been identified (reviewed in Rosenberg et al. 2007).

Due to the opportunistic manner in which disease samples must often be collected, many coral pathogens were identified based upon limited host, geographic, and temporal occurrences of disease. The tendency to group occurrences of coral mortality with similar gross lesion morphologies into named diseases, has often led to the assumption that all instances of a given "disease" are identical. By extension, the practice has propagated the idea that a given pathogen is responsible for all pathologies with similar lesions. Recent evidence, such as the observation of apparent white plague disease in the absence of the published pathogen (Pantos et al. 2003), has brought these assumptions under scrutiny.

The use of PCR has become very commonplace, with most research facilities having access to the required equipment and expertise. Pathogen-specific PCR assays have been extensively utilized for the detection of various human and veterinary pathogens; evolving into a relatively simple and inexpensive detection method. These assays have the advantage of selectively identifying target sequences from a population and provide a route for further confirmation through sequencing of the amplicons.

This study details construction and testing of PCR primers specifically targeting four bacteria previously implicated in coral disease, *Aurantimonas coralicida*: white plague (type II) (Richardson et al. 1998), *Serratia marcescens*: white pox (Patterson et al. 2002), *Vibrio shilonii*: bleaching (Kushmaro et al. 1997), and *Vibrio coralliilyticus*: bleaching and necrosis (Ben-Haim and Rosenberg 2002). Also presented is the application of these primer sets to healthy and diseased corals in the Florida Keys, Puerto Rico, and U.S. Virgin Islands. These assays represent cost-effective mechanisms for rapid, initial screening for the presence of the target microbes.

Materials and Methods

Bacterial Strains

Aurantimonas coralicida WP1 (DSMZ 14790) (Denner et al. 2003), *Serratia marcescens* PDL100 (ATCC BAA-632) (Patterson et al. 2002), *Vibrio shilonii* AK-1 (ATCC BAA-91) (Kushmaro et al. 2001), and *Vibrio coralliilyticus* YB (ATCC BAA-450) (Ben-Haim et al. 2003) were used as target bacteria for primer design, sensitivity, and specificity testing. *Escherichia coli* (ATCC 8739) and strains of *Vibrio* spp., *Photobacterium* spp., and *Shewanella* spp. bacteria isolated from healthy acroporid corals (Polson 2007) were used as additional control strains during specificity testing.

Extraction of DNA

Bacterial cultures were grown on glycerol artificial

sea water (GASW) agar media (Smith and Hayasaka 1982) for 18 hr (except *A. coralicida*, 36 hr). Single colonies were transferred to a PCR tube containing 150 µl of QuickExtract reagent (Epicentre). Each was incubated at 65ºC for 30 min and 98ºC for 16 min with vortexing at each step.

DNA extractions from environmental samples were performed by processing 200 µl of liquid sample (mucus, seawater, or mucus-tissue slurry) or ~0.5-1g of cryo-milled coral fragments using the FAST DNA Spin Kit for Soil (Qbiogene) according to modifications of Webster *et al.* (2003).

Primer Design and PCR Optimization

Sequence data for the 16S rDNA of a given target bacterium, sequences from closely related bacteria, and outlier sequences were aligned using Lasergene MegAlign (DNAStar). Primer sequences were chosen from regions with increased variability between target and comparison sequences. Each primer sequence was checked for specificity by BLAST against GenBank nucleotide database and analysis with the Ribosomal Database Project's (RDP) Probe Match. Candidate primers were checked for interfering secondary structure with Lasergene PrimerSelect (DNAStar).

Temperature gradient PCR was run on each candidate primer pair using DNA from the target bacterium as template. Approximately 10 ng of DNA template (optical density 260 nm) was added to PCR mixture (2mM $MgCl_2$, 0.2 mM dNTP (each), 0.2 µM primers (each), 1.25 U ExTaq Polymerase (Takara)) with the following thermal cycle: 95ºC-5min; 30 cycles: 95ºC-30s, 62ºC±10ºC-30s, 72ºC-60s; 72ºC-10min. PCR products were visualized by electrophoresis on a 1% agarose gels post-stained with ethidium bromide. Annealing temperatures were chosen based on highest annealing temperature producing a consistently visible PCR product.

Selected primer sets (Table 1) were compatible with a uniform annealing temperature of 67ºC. Final conditions for assay: 10-20 ng of DNA were added to 50 µl reactions using the standard mixture and PCR was performed: 95ºC-5min; 30 cycles: 95ºC-30s, 67ºC-30s, 72ºC-60s; 72ºC-10min. Amplicons were visualized by gel electrophoresis (Fig. 1).

Table 1. Coral pathogen targeted primer sets with expected amplicon size. Primer sequences oriented from 5' to 3'.

Target	Name	Sequence
A. coralicida	Ac-995F	TCG ACG GTA TCC GGA GAC GGA T
(500 bp)	UB-1492R	TAC GGY TAC CTT GTT ACG ACT T
S. marcescens	Sm-456F	GGT GAG CTT AAT ACG TTC ATC A
(1040 bp)	UB-1492R	TAC GGY TAC CTT GTT ACG ACT T
V. coralliilyticus	Vc-76F	GTT RTC TGA ACC TTC GGG GAA CG
(940 bp)	Vc-1019R	CTG TCT CCA GTC TCT TCT GAG G
V. shilonii	Vs-457F	GGT ACG TTA ATA GCG TGC TCG
(570 bp)	Vs-1023R	ACC TGC GTC TCC GCT GGC

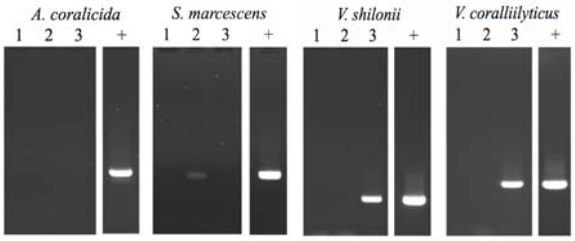

Figure 1. Representative assay results from three samples taken in Biscayne National Park, FL. +: positive control

Primer Specificity

One hundred forty-two samples of coral mucus/tissue or seawater, collected from reefs in the U.S. Virgin Islands National Park (USVINP), St. John, U.S. Virgin Islands, Florida Keys, and southwestern Puerto Rico, were used as material for testing primer specificity. DNA extraction, PCR, and amplicon visualization were performed as described earlier. Suitable PCR bands were excised from agarose gels.

Gel purification was performed using the NucleoSpin Extraction Kit (Clontech). Amplicons were cloned by use of the TOPO TA Cloning Kit (Invitrogen). Plasmids were purified using the QIAprep 96 Turbo kit (QIAGEN) and sequenced with standard M13F and M13R primers.

Assay Sensitivity

GASW broth was inoculated with 1ml of an overnight broth culture of a target microbe or *E. coli*. Flask cultures were incubated at 25ºC with shaking until mid-logarithmic growth phase. Two 25ml volumes were centrifuged at 4ºC for 10 min at 3500 X *g*, washed in filter-sterilized artificial seawater (FASW; 35 ppt), resuspended in 25 ml of FASW, and frozen at -80ºC (mimicking sample collection protocol). Three 1 ml sub-samples of the growth culture were serially (10-fold) diluted in FASW and 100 µl of the 10^3 through 10^6 fold dilutions were spread onto GASW agar, incubated at 25ºC until sufficient colony growth and colony forming units (cfu) determined.

The 25ml volumes were thawed and diluted to 10^6 cfu per ml. Each was then diluted with washed *E. coli* cells (10^6 cfu/ml), reducing concentration of target microbes, but maintaining a background of 10^6 cfu/ml. Fifteen standards were prepared ranging from 1000 to 0.31 cells/µl (extrapolated from cfu/ml data). DNA was extracted from each sample as described for environmental samples and PCR amplifications were conducted as described for assay PCR.

Results

Primer Specificity

The 142 samples (mucus, slurry, milled, and water) screened for the presence of the four target microbes

Table 2. Environmental sample results used in primer specificity testing. Numbers denote positive results by PCR assay for each target microbe.

	Florida Keys	Virgin Islands	Puerto Rico	**Total**
# of Samples	**67**	**24**	**51**	**142**
A. coralicida	4	5	4	**22**
S. marcescens	0	7	13	**11**
V. shilonii	5	0	1	**6**
V. coralliilyticus	4	0	3	**7**

resulted in 46 positive amplifications (Table 2). All amplicons produced from *A. coralicida* primer sets resulted in clone sequences consistent with the target organism. In all cases the highest sequence homology to a characterized strain was to *A. coralicida*.

Amplicons indicating positive matches for *S. marcescens* resulted in closest homology to *S. marcescens* strains by BLAST and RDP Seqmatch. Some sequences, while 100% identical to *S. marcescens* strains, were also indistinguishable from bacteria identified in the BLAST database as *Kluyvera ascorbata*.

Vibrio shilonii amplicons displayed very close homology to *V. shilonii* strains, but it should be noted that strains of *V. mediterranei, V. aestaurianus, V. ichthyaenteri,* and *Listonella anguillarum* (=*V. anguillarum*) often show very high homology to the known *V. shilonii* sequences. Similarly, *V. coralliilyticus* primers resulted in sequences consistently displaying high identity with *V. coralliilyticus*, but often also similar to *V. neptunis, V. brasiliensis,* and *V. shilonii.*

Assay Sensitivity

Amplification of target DNA was, in all cases, possible at concentrations below 1 target cell/µl. *S. marcescens* and *V. coralliilyticus* primers sets produced easily visible bands at the minimum tested concentrations of 0.31 target cells/µl. The *A. coralicida* primer set produced easily visible bands to 5 cells/µl, with detectible product to 0.31 cells/µl. The *V. shilonii* primer set produced properly-sized amplicons (~600 bp) to 0.62 cells/µl. *V. shilonii* primers non-specifically amplified background *E. coli* DNA forming a product well above the anticipated size (~2.5 kb). This product was easily discernible from the target amplicons.

Field Testing

Sixty seven samples were assayed from healthy and diseased acroporid corals at 6 sites from Biscayne National Park, Florida Keys National Marine Sanctuary, and Drt Torugas National Park during a 2003 acroporid mortality event (Williams and Miller 2005). The presence of three of the microbes (*A. coralicida, V. shilonii*, and *V. coralliilyticus*) were indicated in the coral sample set (no instances of *S. marcescens*), while none of the targets were detected in the samples from the surrounding seawater. Sampling location, coral species, sample type (mucus or fragment), and health state did not correlate with the occurrence of any of the microbes.

Acropora spp. corals were sampled at five sites surrounding the island of St. John (USVI) from healthy corals and those exhibiting signs consistent with white pox disease or white band disease. Positive results were obtained from *A. coralicida* and *S. marcescens* (Table 3). While these microbes were present in ten and seven samples respectively, no consistent associations were noted with either white band or white pox like disease states. Sampling location and coral species were also not linked to the occurrence of any of the microbes.

Samples were collected from 51 apparently healthy *Porites astreoides* colonies at eight sites near La Paguera, Puerto Rico. Results indicate the presence of each of the four target bacteria. *S. marcescens* was the most commonly detected, with six of seven colonies at one site (17.93ºN 66.94ºW) and three of six at an adjacent site (17.96ºN 67.02ºW) testing positive for this microbe.

Discussion

Primers were initially tested against a small number of known bacterial cultures, but the

Table 3. Results of primer assay on acroporid corals near St. John, U.S Virgin Islands. Diseased corals were characterized as exhibiting signs consistent with white band disease (WBand) or white pox (WPox), based on field observations.

ID	Health	Species	*A.cor.*	*S.mar.*	*V.shi.*	*V.cor.*
4	Healthy	*A. palmata*				
7	Healthy	*A. palmata*		X		
8	Healthy	*A. palmata*				
9	Healthy	*A. palmata*	X			
3	Healthy	*A. cervicornis*			X	
5	Healthy	*A. cervicornis*			X	
6	Healthy	*A. cervicornis*	X	X		
10	Healthy	*A. cervicornis*				
16	Healthy	*A. cervicornis*				
17	Healthy	*A. cervicornis*	X	X		
11	WPox	*A. palmata*				
13	WPox	*A. palmata*	X			
14	WPox	*A. palmata*				X
15	WPox	*A. palmata*				
19	WPox	*A. palmata*	X			
18	WPox	*A. palmata*	X			
28	WPox	*A. palmata*		X		
30	WPox	*A. palmata*				
12	WBand	*A. palmata*	X	X		
23	WBand	*A. cervicornis*				
24	WBand	*A. cervicornis*	X	X		
26	WBand	*A. cervicornis*		X		
27	WBand	*A. cervicornis*	X		X	
29	WBand	*A. cervicornis*	X			

sometimes crowded phylogenies and abundance of undescribed and/or unculturable phylogenetic neighbors made exhaustive specificity testing in this manner unwieldy and potentially incomplete. The model for testing used here relied on virtual hybridizations against sequences from the RDP and NCBI nucleotide databases, followed by application of the assays to numerous coral samples from multiple locations, dates, species, and health states. All amplicons were validated by DNA sequence analysis. In this manner, the primers were subjected to evaluation against a large number of environmentally relevant microorganisms.

The ability to exhaustively validate the specificity of the *Aurantimonas coralicida* primers is limited by a lack of suitable representatives of this relatively novel and poorly characterized genus (3 members) and candidate family (2 genera). Without a more extensive database of sequences from these taxa it is hard to determine specificity precisely, but based on sequencing of environmental amplicons it appears that only sequences with near 100% identity to the pathogenic strain are amplified using this primer set.

The *S. marcescens* primer set displayed a very high level of specificity, with multiple strains of *S. marcescens* inducing a positive result while no other species of *Serratia* were detected in samples. Identical sequences are deposited in GenBank for some strains of *S. marcescens* and the related species *Kluyvera ascorbata*. The phylogenetic relationships among genera of the family Enterobacteriaceae are poorly defined and overlap between genera has been reported (Pavan et al. 2005). There are no reports of *Kluyvera* spp. associated with any marine organism and only a single *Kluyvera* sp. sequence (DQ316136; unpublished) deposited in GenBank is noted to be from the marine environment (seawater from China), thus the likelihood of a non-specific identification of *K. ascorbata* in coral is exceedingly unlikely.

The genus *Vibrio* is highly subdivided, comprised of 88 species with standing in nomenclature (Euzéby 2009). There is much contention over the criteria used to divide related strains into separate species. Many have 99 to 100% homology over portions (and occasionally the full length) of the 16S rDNA. The issue of differentiating *Vibrio* spp. is further compounded by the sheer number of sequences deposited, the GenBank database contained 5,473 16S rDNA sequences annotated "*Vibrio"* (January 2009). Many of these clones were annotated based solely on similarity of 16s rDNA, thus many are likely misidentified at the species or even genus level. In addition there are countless unannotated clones that are likely *Vibrio* spp. These combined issues make the task of determining specificity among *Vibrio* specific primers very difficult.

Amplicons from *V. shilonii* and *V. coralliilyticus* primer sets produced sequences that were difficult to precisely place phylogenetically. These sequences did however show a high level of specificity, as only slight degeneracy in primer binding would have been expected to produce amplicons from every sample due to the ubiquitous nature of V*ibrio* spp. in the reef environment. These primers only detected sequences that would be expected based on 0 or 1 bp mismatch in the primer binding. It was noted that the *V. coralliilyticus* primers would occasionally amplify sequences homologous to those annotated as *V. shilonii* from environmental samples, but never from DNA extracted from stock cultures of *V. shilonii.* This could be due to some similarity to sequences of certain *V. shilonii* strains, but is very likely due to erroneous annotation of sequences in GenBank. The *V. shilonii* primers never produced amplicons homologous to *V. coralliilyticus* sequences, providing an avenue for detection of false-positives.

Qualitative identification of target microorganisms is classically subdivided into three types of nested tests: presumptive, confirmed, and completed (in order of increasing confidence) (Eaton et al. 1995). A positive result obtained by application of the assays presented here should not be viewed as proof that the target microbe is present. It rather demonstrates that the target bacterium, or one with very close sequence homology, occurs in the sample. Thus the PCR assay should be treated as a "presumptive test", with subsequent sequencing of amplicons acting as the complementary "confirmed test". This confirmed test can provide reasonable certainty that the target is present. The only "completed test" that can be offered is to isolate and characterize the target microbe, followed by laboratory infection studies.

Sensitivity assays indicate the lower detection limits of each assay exceeded 0.62 target cells/µl (620 cells/ml) within a relevant background concentration of approximately 1 x 10^3 non-target cells/µl (1 x 10^6 cells/ml), or 0.06% of the total. These sensitivity levels exceed what would likely be necessary to detect an active pathogen. For instance, *V. shilonii* has been shown to maintain populations in the coral mucus exceeding 1 x 10^6 cells/ml during an infection (Banin et al. 2000). Thus these assays provide detection of target bacteria at what are likely sub-infection levels.

The three case studies presented in this paper, illustrate potential utility of these assays. The 2003 mortality of acroporid corals along the Florida Reef Tract had disease signs, which displayed both differences and similarities to two "white disease", white pox (previously reported in *A. palmata*) and white plague (reported in a large number of non-acroporid species). The application of the primer sets

quickly excluded the previously published white plague and white pox pathogens. These results were later confirmed by extensive culturing and 16S rDNA library sequencing (Polson 2007).

A survey of healthy and diseased acroporid corals from the US Virgin Islands identified a number of both healthy and diseased corals that tested positive for the target microbes. The diseased corals in this sample collection had disease signs that fit the classical descriptions for white band and white pox disease. Interestingly, the white pox samples did not show any correlation with the presence of *S. marcescens*, the reported pathogen from the Florida Keys. This observation underlines the critical point that identical coral disease signs do not necessarily equate to identical etiologies. This survey represents the first confirmation of *Acropora palmata* exhibiting disease consistent with that described as white pox without the association of *S. marcescens.*.

Surveys of *Porites astreiodes* colonies along the southwest coast of Puerto Rico indicated the presence of all targeted microbes. In particular *Serratia marcescens* is noted to occur in approximately 25% of the corals sampled, with greater than 50% abundance at two of the sampling sites. *S. marcescens* is not known to produce disease in *P. astreoides*. While this observation is anecdotal, it does indicate that non-affected coral species may be a potential reservoir for this reported acroporid pathogen.

Each diagnostic primer set demonstrated potential for use in applications ranging from the diagnostic assessment of specific coral disease occurrences to more generalized surveillance of corals. Any application should be undertaken with the caveat that they represent an initial screening tool (a presumptive test) for the presence of target organisms. Despite this limitation, the assay allows for results to be obtained from a large number of samples in a relatively short period of time, quickly narrowing the number of samples on which more intensive investigation may be needed. The utilization of a standardized protocol (sampling, processing, and analysis) will allow data comparisons across laboratories, enabling the combination of disparate sample sets that would not normally be processed together, setting the stage for addressing large-scale questions.

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Coral-associated ammonium oxidizing Crenarchaeota and their role in the coral holobiont nitrogen cycle

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Abstract. Genetic comparison of Archaea associated with the surface mucus of corals from three genera, namely *Acanthastrea* sp., *Favia* sp. and *Fungia* sp. from the Gulf of Eilat, Israel and from Heron Island, Australia were studied. Sequencing of the 16S rRNA gene of the coral-associated microorganisms revealed dominance of Crenarchaeota (79%, on average). In this phylum, 87% of the sequences were similar (≥97%) to the Thermoprotei, with 76% of these being similar (≥97%) to the ammonium oxidizer, *Nitrosopumilus maritimus*. Analysis of archaeal *amoA* sequences obtained from the fungiid coral, *Fungia granulosa*, divided into three clades, all related to archaeal sequences previously obtained from the marine environment. These sequences were distantly related to *amoA* sequences previously found in association with other coral species. Preliminary experiments suggest that there is active oxidiation of ammonia to nitrite in the mucus of *F. granulosa*. Thus, coral-associated Archaea may contribute to nitrogen recycling in the holobiont, presumably by acting as a nutritional sink for excess ammonium trapped in the mucus layer, through nitrification processes.

Key words: Archaea, ammonia, *amoA*.

Introduction

Coral reefs develop in oligotrophic marine environments. The surface of scleractinian corals, the main reef builders, is covered by a layer of mucopolysaccharides. This mucus layer is made up of a gel consisting of an insoluble hydrated glycoprotein (Brown and Bythell 2005) that is constantly secreted by the coral tissue. At the point of contact of the mucus with the surrounding water, a boundary layer is formed (Segel and Ducklow 1982; Ritchie and Smith 2004). Recent research has demonstrated that corals contain large, diverse and specific populations of microorganisms, including Viruses, Bacteria, Archaea, fungi, algae and protozoa that apparently co-evolved with corals (reviewed by Rosenberg et al., 2007). The presence of archaeal groups in association with corals has been reported (Wegley et al. 2004; Kellogg 2004), although the roles assumed by the Archaea in the coral holobiont have not yet been studied.

Archaea comprise four phyla, namely the Crenarchaeota, Euryarchaeota, Korarchaeota, and Nanoarchaeota. In the ocean, planktonic Crenarchaeota are most abundant (Fuhrman 1992; Karner et al. 2001). Although Crenarchaeota constitute about 20% of the total marine picoplankton biomass world-wide (Karner et al. 2001), their roles in the marine biogeochemical cycle have not been thoroughly explored (Wuchter et al. 2006). Until recently, Archaea were considered to be uncommon partners for invertebrate symbioses. Archaea were found in association with sponge species (Preston et al. 1996; Webster et al. 2001; Pape et al. 2006), although the ecological functions assumed by these sponge-linked microorganisms remain unknown.

Wegley et al. (2004) showed by fluorescent in situ hybridization (FISH) that Archaea on the coral, *Porites astreoides*, accounted for nearly half of the prokaryotic community, implying that Archaea are abundant, diverse, and potentially important components of the coral holobiont.

Corals obtain and assimilate nitrogen through predation (Piniak et al. 2003). Additional nitrogen can be obtained through direct absorption from the surrounding water (Muscatine and D'Elia, 1978; Badgley et al., 2006), by integration via associated microorganisms, such as nitrogen-fixing cyanobacteria (Lesser et al. 2004), and from microorganisms trapped in the coral mucus (Wild et al. 2004). Nitrogen metabolized by the corals leads to the production of ammonia that can be excreted into the environment.

Aerobic oxidation of ammonia by some microorganisms is catalyzed by ammonia

monooxygenase (AMO), an enzyme that converts ammonia to hydroxylamine, which is further converted to nitrite by hydroxylamine oxidoreductase (Nicol and Schleper 2006). A time series study in the North Sea revealed that the abundance of archaeal *amoA* was 1 to 2 orders of magnitude higher than those of *amoA* from nitrifying bacteria. Sea water incubated with ammonia was dominated by a single member of the crenarchaeotal phylogenetic cluster showing 99% sequence identity over the nearly complete 16S rRNA gene of the nitrifying Crenarchaeote, *Nitrosopumilus maritimus* (Wuchter et al. 2006). *N. maritimus* is a marine chemolithtotroph which aerobically oxidizes ammonia to nitrite with near stoichiometric conversion, using ammonia as its sole energy source (Könneke et al. 2005).

Wafar et al. (1990) suggested that nitrification occurs at significant rates in living coral colonies. The authors showed that nitrifying coral-associated microorganisms can vary from 4 to 260 x 10^3 cells per mg coral tissue. Recently putative archaeal *amoA* genes encoding ammonia monooxygenase subunit A were retrieved from several corals (Beman et al. 2007). Indeed, while archaeal *amoA* sequences were obtained from different species of coral-associated Archaea, no bacterial *amoA* sequence could be amplified from any of these samples.

In this report, we study coral-associated Archaea from three genera of corals (*Acanthastrea* sp., *Favia* sp. and *Fungia* sp.) from the Red Sea, Israel with those from Heron Island, Australia concentrating in Crenarchaeota. Based on our findings, the possible role of coral-associated Archaea in the coral holobiont is discussed.

Material and Methods

Samples of the hermatypic corals, *Fungia* sp., *Favia* sp. and *Acanthastrea* sp., were collected at depths of 5-10 m at Heron Island GBR Australia (23º26 'S, 151°54 'E), in July, 2006, and near the Inter-University Institute for Marine Science in the Gulf of Eilat (29°51 'N, 34°94 'E), Red-Sea, in June, 2007. Within one hour of collection, the corals were placed in aquaria filled with running sea water. On the same day, mucus was sampled from the corals using sterile bacteriological loops. Sea water from Eilat was collected by opening a sterile container on the same dives. Four additional *Fungia granulosa* individuals were collected in December, 2007 for mucus collection, for NO_2 production experiments. This was carried out by inverting the corals on a funnel for 2 min. Mucus for DNA extraction was sampled from two of the *F. granulosa* corals using bacteriological loops, as previously described (Barneah et al. 2007).

F. granulosa mucus (2 ml) was filtered through 0.1 μm filter. The filters were then placed in glass flasks with 10 ml sterile sea water and 1mM ammonium chloride at 22-25ºC and incubated with shaking for up to 28 days. Since streptomycin inhibits the growth of nitrifying bacteria (Könneke et al. 2005), to eliminate the activities of bacterial ammonia oxidation, two flasks were incubated with 500 mg L^{-1} of streptomycin. Eight milliliters of filtered mucus topped with 2 ml sterile sea water containing 1 mM ammonium chloride served as control. NO_2 was measured after 0, 2, 15 and 28 days, according to standard methods for the examination of water and wastewater (Clesceri et al. 1998).

Genomic DNA from the mucus samples was extracted by a PowerSoil purification kit (Mo Bio Laboratories Solana Beach, CA. USA), and stored at -20°C. To extract genomic DNA from the water column, 2 L of sea water collected from the same depths were passed through sterile 0.2 μm filters and the DNA was extracted as above. Total DNA was PCR-amplified using specific archaeal 16S rRNA gene modified primers; Arch-21F (5'-TTCCGGTTG ATCCYGCCG-3'), obtained from DeLong (1992) and Arch-915R (5'-GTGCTCCCCCGCCAATTC-3'), taken from Amann et al., (1995). An initial denaturing step of 4 min at 95°C was followed by 30 cycles of the following program pattern: 94°C for 30 sec, 54°C for 30 sec, and 72°C for 70 sec. The procedure was completed with a final elongation step at 72°C for 30 minutes. Archaeal *amoA* gene fragments were amplified using Arch-amoA-F (5'-STAATGGTCTGGCTTAGACG-3') and Arch-amoA-R (5'-GCGGCCATCCATCTGT ATGT-3') primers and conditions as previously described (Francis et al. 2007; Beman et al. 2007).

The gel-purified PCR products were cloned into the PCRII-TOPO- A cloning vector, as specified by Invitrogen (Carlsbad, CA) and transformed into calcium chloride-competent *Escherichia coli* HD5a cells. More than 50 clones from each sample were amplified using M13-F and M13-R primers and sequenced, using the Arch-21F primer. All sequences were first compared with those in the GenBank database with the basic local alignment search tool (BLAST) network and check for chimeric sequences using Bellerophon (Huber et al. 2004).

Results

A total of 424 clones derived from 8 coral samples (>50 clones from each library) displayed a prevalence of crenarchaeotal sequences (79%), as compared to euryarchaeotal sequences (21%) (Fig. 1). Red-Sea water displayed opposite prevalence with dominants of Euryarchaeota (63%).

BLAST analysis revealed that of the coral-drived crenarchaeotal sequences, 87% were similar (≥97%) to the class Thermoprotei. Of this group, 76% were

similar (≥97%) to sequence of *Nitrosopumilus maritimus* (DQ085097) (Fig. 1), a marine Archaea that oxidizes ammonia Könneke et al., (2005).

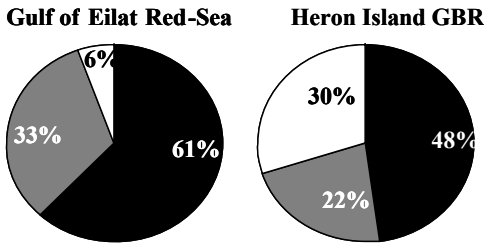

Fig. 1: Archaea distribution by sample location. Crenarchaeota similar to *Nitrosopumilus maritimus* (≥97%, FastGroup II) (black), other Crenarchaeota (gray) and Euryarchaeota (white).

Analysis of the archaeal *amoA* from two *F. granulosa* corals, Rs-Fg1 and Rs-Fg2 (24 sequences in each clone library), collected from the Red Sea, yielded three different clades, all related to sequences retrieved from the marine environment. Part of the nitrification cycle of the mucus-associated microorganisms was demonstrated by nitrite production.

Nitrification measurements of *F. granulosa* mucus-derived microbiota incubated with 1 mM ammonia revealed an increase of 50% after 15 days and a 9-fold increase after 28 days (Table 1). Production of nitrite after 28 days of incubation in a streptomycin-amended medium (designed to inhibit nitrifying bacteria) was slightly lower than in non streptomycin-amended samples.

Table1: NO_2 production by *F. granulosa* coral mucus-associated microorganisms during 28 days.

Days NO_2 (ppm)	0	2	15	28
Control	0	0	0.037	0.096
Coral mucus microorganisms	0	0	0.1186	1.076
Coral mucus microorganisms + streptomycin	0	0	0.118	0.856

Discussion

Phylogenetic analysis of archaeal 16S rRNA gene sequences retrieved from three corals species from the Red Sea (*Fungia* sp., *Favia* sp. and *Acanthastrea* sp.) displayed high similarity (up to 100%) to archaeal sequences retrieved from the same coral genera from the GBR. 67% of the 424 sequences from those locations were closely related (≥97%) to sequences derived from three scleractinian corals from the Virgin Islands (Kellogg 2004), implying the existence of a general coral-archaeal symbiotic association.

In general, the Crenarchaeota sequences were most abundant in coral samples from the Red Sea than in GBR (Fig. 1). A higher abundance of Crenarchaeota (73%) over Euryarcaeota (27%) was also observed by Wegley et al. (2004). By contrast, Kellogg (2004) reported a dominance of Euryarcaeota (average of 80%) in scleractinian corals from Carribian. This may a biogeographic distinction, or due to differences in sample collection methods.

The prevalence of Euryarchaeota over Crenarchaeota in the sea water sample was previously reported with water samples from oceanic photic zones (Delong et al. 2006; Delong 2007). This result strengthens the premise that conditions in the mucus differ from those in the surrounding medium.

Interestingly, almost all (87%) coral Crenarchaeota sequences analysed by BLAST were most closely related (≥97%) to the Thermoprotei class and 76% of these sequences were highly similar (≥97%) to that of *Nitrosopumilus maritimus* (DQ085097), whereas only 27% of the sea water-associated archaeal clone library sequences were similar to this strain. *N. maritimus*, isolated from the rocky substratum of a tropical marine tank, is an autotrophic crenarchaeote that is able to obtain its energy by oxidizing ammonium, producing nitrite as the end product (Könneke et al. 2005; Delong 2007). Positive correlations between the abundance of Crenarchaeota and ambient nitrite concentrations were observed in the Arabian Sea (Sinninghe et al. 2002) and in Santa Barbara (Murray et al. 1999). It is possible that the coral mucus layer is rich in ammonium and other metabolic by-products of corals, providing a rich nutritive source for these microorganisms.

Though ammonium can be utilized by coral zooxanthellae, very high levels may be detrimental to the coral holobiont. This may be due to the fact that high ammonium levels cause an inhibition of carbohydrate assimilation by algae (Azov and Goldman 1982) or affect other metabolic parameters, such as an inhibition of nitrate uptake (Badgley et al., 2006). It is feasible that mucus-associated Archaea act in the coral holobiont as a sink for excess ammonia, recycling it through nitrification during the day, when oxygen is in excess due to photosynthesis of the zooxanthella symbionts.

I this study we, screen the archaeal *amoA* gene in the Red Sea *Fungia* sp. Results showed that coral-associated archaeal *amoA* genes clustered into three clades, all related to sequences obtained from the marine environment. Surprisingly, *amoA* genes of coral-associated Archaea from Bermuda (Beman et al., 2007) and the *amoA* gene of *N. maritimus* created a separate cluster with very low similarity (79%-85%) to the *amoA* sequences from our coral-associated

Archaea .This may be due to the fact that Archaea from the Bermuda were from corals that were not in as close association to the substrate as the Red Sea fungiids. On the other hand, most of our Crenarchaeota 16S rRNA gene sequences were closely related (≥97%) to *N. maritimus*. These differences may be explained by the choice of genes for comparison, namely the highly conserved 16S rRNA gene (Gutell et al., 1994) and the functional and variable *amoA* gene.

The 16S rRNA gene library of coral-associated Archaea showed that at least 50% of the clones were similar (≥97%) to ammonium oxidizer, *N. maritimus*. To further confirm the presence of ammonia oxidizing Archaea, NO_2 production by coral mucus microorganisms was investigated (Table 1) using streptomycin, an antibiotic that affects Bacteria but not Archaea (Könneke et al., 2005). Results showed an increase in nitrification in the mucus of *F. granulosa* throughout the experiment. Moreover, there was only a slightly lower production of nitrite after 28 days of incubation in a streptomycin-amended medium, as opposed to non-amended samples. These results provide evidence for the possible widespread presence of ammonia oxidizers in coral reefs. Similarly, Wuchter et al., (2006) showed that the addition of nutrients to North Sea water was followed by an enrichment of Crenarchaeota and was correlated with the concomitant decrease of ammonia and increase of nitrite concentrations.

Nutrients from the environment have to move across a physical boundary layer, as well as through the mucus layer, to reach the coral tissue. Nutrients from the coral tissue must pass an opposite route to reach the environment. Such movement of nutrients is governed by a very slow diffusion rate (Segel and Ducklow, 1982). It is likely that a microbe growing on the mucus surface has access to dissolved nutrients before the coral does and, conversely, has access to coral-borne nutrients before the planktonic microorganism population does. Therefore, the coral mucus may be a better living substrate for ammonia-oxidizing Archaea than the water column because these Archaea would encounter ammonia before planktonic organisms and at higher concentrations.

We, therefore, suggest the following role for mucus-associated Archaea in the coral holobiont nitrogen cycle. During the day, the mucus conditions are oxic, and Archaea similar to *N. maritimus* oxidize ammonia to nitrite, which may be assimilated into the coral. At night, the mucus conditions are anoxic and microorganisms, like the archaeal H. salifodinae, may convert nitrite into nitrogen via denitrification pathways. This implies that Archaea play an essential role in coral reef and coral holobiont physiology.

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Coral mucus bacteria as a source for antibacterial activity

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Abstract. In the oligotrophic marine environment there are ecological niches rich in nutrients and diverse in bacterial populations. One such niche is the coral surface mucus layer. Interactions amongst microorganisms found in coral mucus may be symbiotic or competitive; competing over space and food. It has been hypothesized that the microbial communities found on the coral surface may play a role in the coral's defense, possibly through the production of antimicrobial substances. To find potentially active compounds produced by coral-mucus bacteria, over 200 selected microorganisms isolated from the mucus layer of a number of coral species were grown using agar plating technique. Screening for antibacterial substances was performed using overlay and drop techniques, and antibacterial activity was tested against indicator microorganisms. Results indicate that more than 20% of cultivable mucus-associated bacteria originating from hard corals demonstrated antibacterial activity. Isolates obtained from hard corals demonstrated higher percentage of activity than soft corals' isolates. Isolates related to the genera *Vibrio* and *Pseudoalteromonas* demonstrated high activity against both gram-positive and gram-negative bacteria. These results demonstrate the existence of microorganisms with antibacterial activity on the coral surface, indicating that they may play a role in protecting the coral host against pathogens.

Key words: Antibacterial activity, Coral mucus, Mucus-associated bacteria.

Introduction

The coral mucus layer serves as an ecological niche rich in nutrients and diverse in bacterial populations. However, the exact role of these microorganisms in the coral holobiont is yet to be determined. Such interactions may be symbiotic or competitive; competing over space and food. Rohwer et al. (2002) hypothesized that the microbial community found on a coral's surface may play a role in the coral's defense mechanism, possibly by occupying niches or through the production of antimicrobial metabolites.

Various studies have reported antimicrobial activity of extracts from marine organisms such as sponges (Kelman et al. 2001), soft corals (Harder et al. 2003, Kelman et al. 1998) and scleractinian corals (Geffen and Rosenberg 2005, Koh 1997). Kelman et al. (2006) reported that the majority of extracts from six soft corals demonstrated high antimicrobial activity against marine bacteria isolated from surrounding seawater, as opposed to extracts from six stony corals exhibiting little or no antimicrobial activity. Ritchie (2006) found that 20% of cultured bacteria from the mucus layer of the coral *Acropora palmata* displayed antimicrobial activity, and that a novel mucus-mediated medium was found to be selective for isolates that produce antibiotics. Thus, the presence of microbial populations on the mucus surface of various invertebrates may play a part in their defense strategy.

Material and Methods

Samples were collected from coral mucus layer, as well as from sea water and sediment around the corals, from the Gulf of Eilat. Among coral samples collected were seven hard corals (*Platygyra sp.*, *Porites sp.*, *Fungia granulosa*, *Favia sp.*, *Stylophora sp., Pocillopora sp.* and *Turbinaria sp.*) and two soft corals (*Rhytisma fulvum fulvum* and *Xenia sp.*). Mucus samples were collected from apparently healthy corals from the upper portion of the coral colony or polyp, and the collection procedure was carried out as described previously by Barneah et al. (2007). Serial dilutions were performed using autoclaved artificial sea water followed by spreading over marine agar plates at 50% and 10% concentrations.

Isolates were screened for antibacterial activity using two techniques: overlay with soft agar and drop technique. Screening was performed against common indicator bacteria, including *Bacillus cereus*, *Escherichia coli*, *Serratia marcescens* and *Staphylococcus aureus*, as well as the marine pathogen *Vibrio coralliilyticus*. The first method is a modification of the overlay technique described by Geis et al. (1983), and involved pouring soft agar mixed with indicator bacteria over marine agar plates containing 5-week old colonies of marine isolates from coral mucus. For this method, only *B. cereus*

and *E. coli* served as indicator bacteria. Antibacterial activity was defined by measuring the radius of the inhibition zone of the growth of indicator bacteria around the colony after 12 hours of incubation. The drop technique involved incubation of marine isolates in 100% marine broth for 72 hours at 26°C with agitation (120 rpm). After sufficient growth, 10 µl of the marine isolates were placed on agar plates containing 120 µl of an overnight culture of indicator bacteria spread as a lawn, followed by incubation for 12 hours at the optimal temperature for that indicator. Isolates obtained from sea water and sediment were used as negative controls for antibacterial screening, using the drop technique. Supernatant, produced by filtration of marine isolate cultures through sterile 0.2 µm filters, was also tested for antibacterial activity using this technique. Activity was detected by the formation of an inhibition zone where the drop was placed, and recorded qualitatively based on degree of transparency.

Isolates' DNA was extracted and 16S rRNA gene fragments were PCR amplified using 8f and 1512r primers. Sequences were then compared with the GeneBank database (NCBI Blast), and a Phylogenetic analysis was performed using MEGA 3.1 software.

Results

Over 20% of cultivable bacteria isolated from mucus demonstrated activity against indicator bacteria. Seventy eight isolates originating from hard corals were screened for antibacterial activity using the overlay technique. Nineteen of these isolates (24.3%) demonstrated activity against indicator bacteria. The percentage of active isolates from each coral species, as determined using the overlay technique, is presented in Fig. 1. The highest percentage of active isolates originated from *Pocillopora sp.* and *Platygyra sp.* (44% and 38%, respectively).

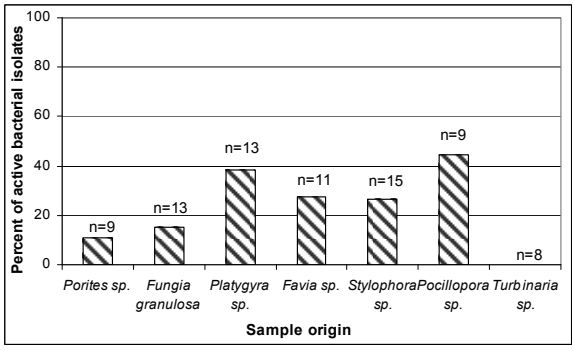

Figure 1: Percentage of active isolates obtained from hard corals. Antibacterial activity was detected using the overlay technique as described in Materials and Methods. n = number of screened bacterial isolates.

146 isolates from soft and hard corals, as well as sea water and sediment, were screened for antibacterial activity using the drop technique. Out of 84 isolates originating exclusively from hard corals, 21 (25%) demonstrated antibacterial activity. However, among 22 isolates obtained from soft corals, only 3 (13.6%) were found to be active. Sediment isolates did not show any antibacterial activity, while only one out of 14 (7%) sea water isolates was found to be active (see Fig. 2). The highest percentage of active isolates originated from *Favia sp.* (42%).

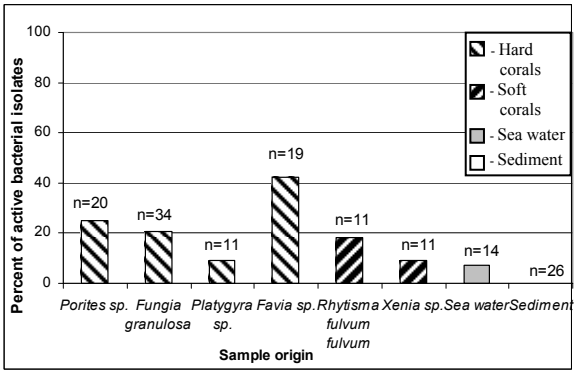

Figure 2: Percentage of active isolates obtained from hard and soft corals, sea water and sediment. Antibacterial activity was detected using the overlay technique as described in Materials and Methods. n = number of screened bacterial isolates.

16S rRNA genes of 46 isolates were sequenced and compared to the NCBI Genebank database. Thirty-eight out of these isolates demonstrated antibacterial activity using either of the screening techniques. Most of the isolates belonged to the *γ-proteobacteria* class (78%), while the rest belonged to the *Firmicutes* (15%) and *Actinomycetales* (7%) phyla. Amongst the *proteobacteria*, isolates related to the genera *Vibrio* and *Pseudoalteromonas* were the most abundant and demonstrated high activity against both gram-positive and gram-negative bacteria.

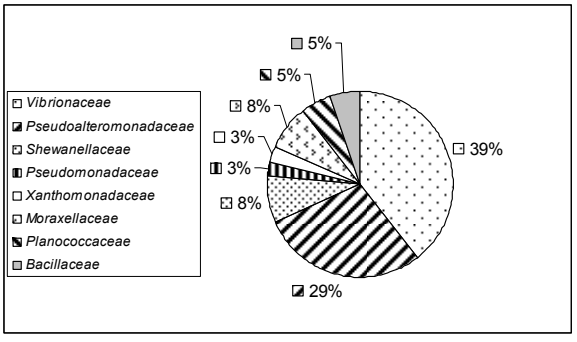

Figure 3: Percent of active isolates from total active isolates sequenced, distributed to each family taxa.

Isolates related to the genera *Shewanella* and *Acinetobacter* only demonstrated activity against gram-positive indicator bacteria. Gram-positive isolates (*Bacillus*, *Planomicrobium*) demonstrated

lower activity, primarily against other gram-positive bacteria. The distribution of active isolates sequenced according to family taxa is presented in Fig. 3.

Discussion

This study tested for antibacterial activity among cultivable coral mucus-associated bacteria and demonstrated that 20-25% of coral mucus cultivable bacteria display antibacterial activity.

Over 230 bacterial isolates obtained from the marine environment of the Gulf of Eilat were screened for antibacterial activity. In general, coral mucus-associated bacteria demonstrated a higher percentage of activity than sea water or sediment isolates. Antibacterial activity detected in isolates originating from hard corals was higher than activity demonstrated by soft coral isolates (25% and 13.64%, respectively). Sea water and sediment isolates demonstrated little or no antibacterial activity.

These results are in agreement with the findings presented by Ritchie (2006), according to which 20% of cultivable bacteria obtained from the mucus layer of the coral *Acropora palmata* demonstrated antimicrobial activity against indicator bacteria. According to Kelman et al. (2006), soft coral extracts were found to be more active than hard coral extracts. The authors also suggested that there are different mechanisms of antimicrobial activity against pathogens involved in scleractinian corals. This also may suggest that production and secretion of antimicrobial compounds by mucus-associated bacteria is part of the scleractinian coral's defense strategy against pathogens (Rohwer et al. 2002; Ritchie 2006; Reshef et al. 2006).

Acknowledgement

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$\delta^{13}C$ and $\delta^{15}N$ values for branching corals in the Berau Marine Conservation Area in East Kalimantan, Indonesia

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Abstract. Carbon and nitrogen isotope ($\delta^{13}C$ and $\delta^{15}N$) values in coral tissue and zooxanthellae of the coral reef species *Porites sp., Seriotophora sp.* and *Stylophora sp.* from the Berau Marine Conservation Area, East Kalimantan, Indonesia were measured. Coral samples were collected during both the dry season (August 2006) and the rainy season (November 2007). Most of the $\delta^{15}N$ values of the coral tissue during the dry season were heavier than those of the zooxanthellae. This result indicates that the zooxanthellae must be the main nutrient source for the coral tissue. However, the majority of $\delta^{15}N$ values for the coral tissues during the rainy season had similar or even lighter values than those of the zooxanthellae, suggesting that during the rainy season corals also utilize particulate organic matter (POM) as a food source.

Key words: $\delta^{13}C$, $\delta^{15}N$, coral tissue, zooxanthellae, particulate organic matter (POM)

Introduction

The Berau Marine Conservation Area (MCA), East Kalimantan, Indonesia has the third highest coral diversity (507 species) in the world (Turak 2003). This area, which consists of 31 islands, is an important coral habitat and a part of the Coral Triangle, the center of tropical marine fish and invertebrate biodiversity. The Berau MCA is also known as the largest rookery for the green turtle in Southeast Asia and a manta ray habitat, as well as including the unique Kakaban Lake with its stingless jellyfish (Wiryawan et al. 2005).

Currently, coral reefs in the Berau MCA are under stress from the combined impact of human exploitation, physical disturbance, pollution, and increased sediment (Turak 2003; Wiryawan et al. 2005). Conversion of mangrove forest to shrimp ponds in the estuary areas, coal mining and loading along the Berau river, and conversion of land for agricultural use and logging in the highlands have been occurring for a long time. All of these human activities could lead to higher sediment loads in the Berau river and ultimately affect the condition of the reefs, especially those located nearest the river mouth.

Data from the EU's Berau Forest Management Project (BFMP, Obidzinski and Andrianto 2005) noted that during 3 years (1997-2000), Berau lost approximately 127,500 ha of forest. In the following six years, from 2000 to 2006, Berau lost 41.1% of protected forest (353,775 ha in 2000 to 208,374 ha in 2006); while fixed forest production decreased from 758,049 ha to 589,567 ha, a reduction of 22.2% (Anonymous 2007). All this deforestation, from both legal and illegal logging activities, has a tremendous big impact on the condition of soil and water resources (Obidzinski and Andrianto, 2005).

The average river discharge is approximately 1,200 m^3/s into the coastal waters. The sediment concentration in the river water was 50 mg/l on average and Buschman (2007) estimated that for every year approximately 2 metric tons of sediment are exported to the Berau coastal area.

The main objective of this study is to understand the effects of the sediment discharge on the condition of the coral reef using $\delta^{13}C$ and $\delta^{15}N$ analysis. Analysis of $\delta^{13}C$ and $\delta^{15}N$ have been successfully used to trace the input of organic matter, assuming that the $\delta^{13}C$ of the consumer is correlated with the $\delta^{13}C$ of their diet and that the $\delta^{15}N$ value of the consumer is heavier than the $\delta^{15}N$ value of their diet (Peterson and Fry, 1987, Yamamuro and Kayanne, 1995)

All reef-building hard corals contain zooxanthellae which live symbiotically within the tissue of the corals and provide energy and nutrients. Generally, coral animals obtain most of their nutrients from the zooxanthellae, causing the $\delta^{15}N$ value of the coral to be heavier than the zooxanthellae. However, in turbid water conditions, the zooxanthellae may fail to provide enough nutrients due to a turbidity dependent reduction in products of photosynthesis, resulting in a change in the relative $\delta^{13}C$ and $\delta^{15}N$ values for the coral and zooxanthellae. In this study, we compared isotopic values of zooxanthellae and

coral tissue collected from high sedimentation coastal areas and clear water offshore areas at two different seasons and depths.

Materials and Methods

Coral samples were collected both during the dry season, in August 2006, and the rainy season, in November 2007, from three sites, designated Locality-1 (nearest the Berau River mouth at the Rabu-rabu and Panjang Islands), Locality-2 (an intermediate distance from the river mouth, at Derawan Island), and Locality-3 (farthest from the river mouth, at the Semama and Sangalaki Islands) (Fig.1-A and B). Samples were collected at two different depths, 3 meters and 10 meters.

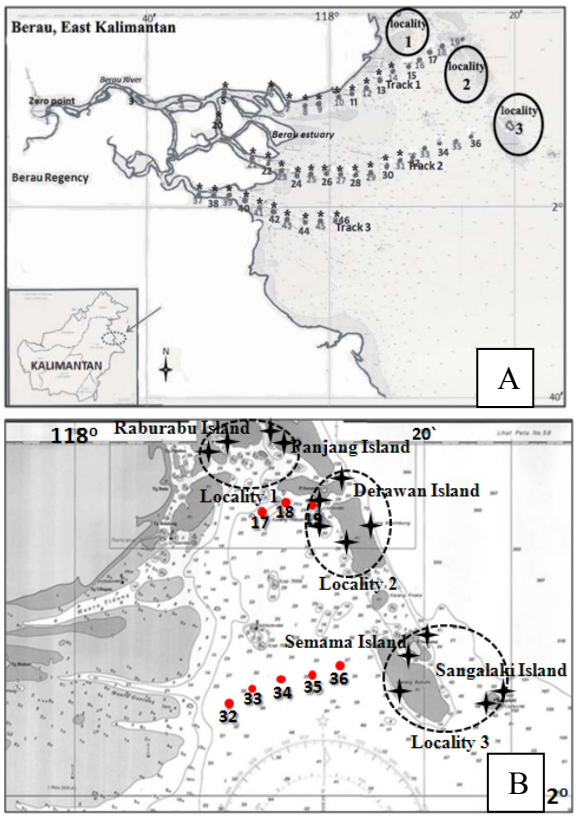

Figure 1. (A) Map of the study area showing the sampling points for sediment and plankton along track 1, track 2, and track 3. (B) Map of coral reef sampling areas at Locality 1, Locality 2, and Locality 3. ✦ indicates a dive point.

Zooxanthellae were separated using a standard ethanol and sonication method. Coral samples were treated with an ultrasonic generator for 10 min and centrifuged at 3000 rpm for 10 min (Piniak and Lipschultz 2004). The resulting pellet of zooxanthella was decalcified using HCl to remove any carbonates and then rinsed twice with DDW. Coral tissue was obtained by decalcification method. Sample was ground into coarse fragments and placed in a cellulose tube. The tube was placed in a beaker containing 0.2 N HCl on a stirrer (Horai et al. 1989). Decalcified zooxanthellae and the tissue were then centrifuged and freeze-dried overnight.

In addition, water parameters such as salinity, temperature, turbidity, pH, and dissolved oxygen (DO) were monitored using a Water Quality Checker (Horiba) along three track lines, track 1, track 2, and track 3 representing the northern, middle, and southern parts of the river effluent, respectively. Water parameters were also measured near the coral reefs (Fig.1). Bulk Particulate Organic Matter (POM) samples were collected from surface water using plankton nets with 100 µm and 310 µm mesh sizes. POM was centrifuged at 2500 rpm. The resulting pellet was decalcified by HCl treatment, rinsed twice, and freeze-dried overnight. Zooplanktons were disaggregated from the POM samples using a binocular. Aliquots (0.8±0.05 mg) of all samples were placed in tin capsules for isotopic measurements.

A total of 132 coral reef and 160 POM samples were measured using an ANCA mass spectrometer. The $\delta^{13}C$ and $\delta^{15}N$ values are expressed in ‰ as defined by the following equation:

$\delta^{13}C$ or $\delta^{15}N$ (‰) = $(R_{sample}/R_{standard} - 1) \times 10^3$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Measurement errors were 0.1‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$ and data are reported relative to Pee Dee Belemnite (PDB) and Air standards, respectively.

Results and Discussion

Water quality tests

The water salinity ranged from 32.5±1.4‰ in the rainy season to 35.8±0.8‰ in the dry season. The average salinity at the bottom of the water column was slightly higher than the surface water salinity in both seasons. Turbidity values (Table 1) measured in Locality 1 were higher than all others.

Table 1. Average water parameters at the reef areas.

Locality	Dry season (Aug 2006)		Rainy season (Nov 2007)	
	surface	bottom	surface	bottom
Turbidity (NTUs)				
Locality 1	0.8±1.0	2.5±2.5	1.5±1.3	2.5±2.6
Locality 2	0	0.3±0.5	0	0.5±1.0
Locality 3	0	0	n.d	n.d
pH				
Locality 1	8.4±0.1	8.4±0.0	8.0±0.1	7.9±0.0
Locality 2	8.4±0.1	8.4±0.1	8.0±0.0	8.0±0.0
Locality 3	8.4±0.1	8.4±0.1	n.d	n.d
DO (mg/l)				
Locality 1	6.3±0.2	6.4±0.1	5.5±0.0	5.5±0.0
Locality 2	6.7±0.9	6.8±0.9	5.7±0.1	5.6±0.1
Locality 3	6.8±1.2	6.8±1.1	n.d	n.d

Turbidity at Locality 1 was slightly higher during the rainy season than in the dry season, which may have

been the result of heavy rainfall and higher river discharge, and, consequently, pH and DO values were lower in the rainy season.

These results correspond to the difference between the average rainfall rate during the dry season of August 2006 (83.7 mm) and the rainy season of November 2007 (235.8 mm) (Anonymous 2008).

δ¹³C and δ¹⁵N of coral tissue and POM

The range of $\delta^{13}C$ and $\delta^{15}N$ values of coral tissue and zooxanthellae from three branching coral reefs are shown in Fig. 2. The average $\delta^{13}C$ values of coral tissue and zooxanthellae of the *Porites sp.* were less negative than those of *Stylophora sp.* and *Seriotophora sp.*. The average $\delta^{15}N$ values for coral tissue in all the branching corals was always heavier than those of the zooxanthellae. However, analysis of variance revealed that there were no significant differences in $\delta^{15}N$ value among species ($p > 0.05$).

The average $\delta^{13}C$ value of POM near the coral reefs was -18.5±1.3‰. The average $\delta^{13}C$ value of POM during the dry season (-18.6±0.8‰) was slightly lighter than during the rainy season (-18.4±1.6‰). Almost all $\delta^{13}C$ values of POM depend on the marine planktonic organic carbon (-19.5‰; Chisholm and Koike, 1996) rather than the coral ecosystem (-12 to -13‰; Muscatine et al. 1989).

$\delta^{15}N$ values of POM ranged from 0.9‰ to 6.3‰ with an average of 4.2±1.6‰. The average $\delta^{15}N$ value during the dry season (5.5±0.9‰) was heavier than during the rainy season (3.3±1.4‰). The shift in $\delta^{15}N$ values of POM at each locality is shown in Fig. 2. The decrease in $\delta^{15}N$ value of POM during the rainy season may be caused by denitrification processes which can reduce $\delta^{15}N$ values by 20-30% (Montoya 2007).

As shown in Fig. 2, the average $\delta^{15}N$ values of POM during the rainy season at each locality more depleted than those collected during the dry season. Hence, $\delta^{15}N$ values of POM during dry season were heavier than $\delta^{15}N$ values of coral tissue. In contrast, $\delta^{15}N$ values of POM during the rainy season were lighter than $\delta^{15}N$ values the coral tissue, which may reflect an increase in primary production

δ¹³C and δ¹⁵N values differ with depth

Muscatine and Kaplan (1994) noted that the $\delta^{13}C$ and $\delta^{15}N$ values of zooxanthellae and coral tissue generally become more depleted in most species with increasing depth. Our data (Fig. 3) exhibited a similar trend between samples collected at a depth of 3 m or 10 m, but there were no significant differences in isotopic values.

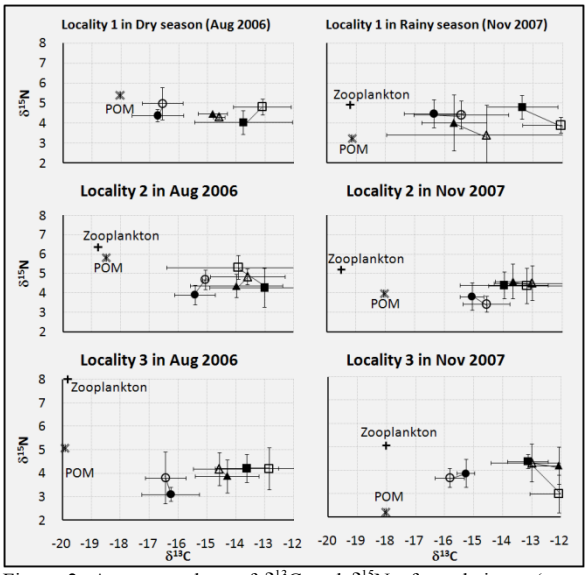

Figure 2. Average values of $\delta^{13}C$ and $\delta^{15}N$ of coral tissue (open symbols), zooxanthellae (filled symbols), and POM and zooplankton at each locality during the dry season (August 2006) and the rainy season (November 2007). □ *Stylophora sp.* ○*Seriotopora sp.* △ *Porites sp.*

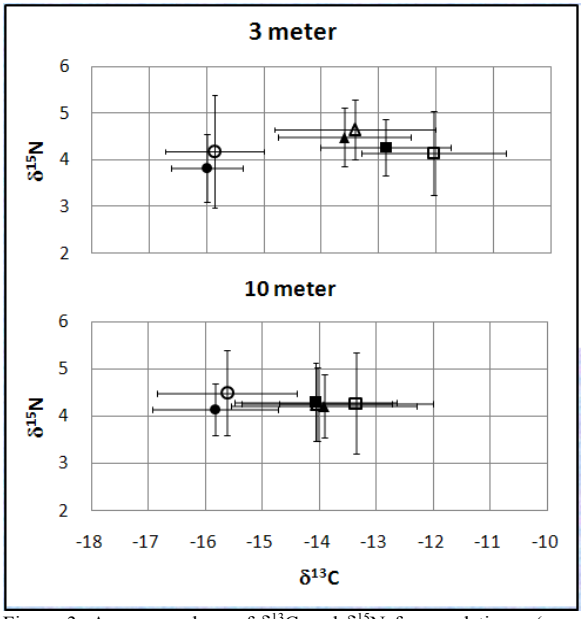

Figure 3. Average values of $\delta^{13}C$ and $\delta^{15}N$ for coral tissue (open symbol) and zooxanthellae (filled symbol) at a depth of 3 meters and 10 meters. □ *Stylophora sp.* ○*Seriotopora sp.* △ *Porites sp.*

δ¹⁵N differences between coral tissue and zooxanthellae

During the dry season, the average $\delta^{15}N$ value for coral tissues was 4.8±0.7‰ and that for zooxanthellae was 4.2±0.6‰. During the rainy season, the average $\delta^{15}N$ values of coral tissues and zooxanthellae were 4.1±1.0‰ and 4.4±0.7‰, respectively.

The $\delta^{15}N$ values of consumer tissues are known to correlate with their diet. The $\delta^{15}N$ values are observed to increase with increasing trophic level. The $\delta^{15}N$ difference between coral tissue and zooxanthellae indicates that the zooxanthellae play a role in providing nutrients to the coral. During the dry season, most of the coral tissue samples had $\delta^{15}N$ values that were heavier than those of the zooxanthellae. This result indicates that the zooxanthellae must be the main source of nutrients for the coral. However, measurement of samples collected during the rainy season revealed that the $\delta^{15}N$ values of the corals were not always heavier than those of the zooxanthellae. The $\delta^{15}N$ values had a tendency to be similar to, or even lighter than, those of the zooxanthellae.

Fig. 4 shows the mean differences in $\delta^{15}N$ ($\Delta\delta^{15}N$) between coral tissue and zooxanthellae at each locality. During the dry season, the mean differences in $\delta^{15}N$ were always positive, while during the rainy season the differences were negative. The $\Delta\delta^{15}N$ is statistically different between the dry season and the rainy season at the 95% confidence interval (ANOVA, $p < 0.000$). This result suggests that during the rainy season the corals utilize food sources other than the zooxanthellae. Since November is the beginning of the rainy season and algal blooms, the corals are likely to be using POM as their main source of organic nutrient compounds

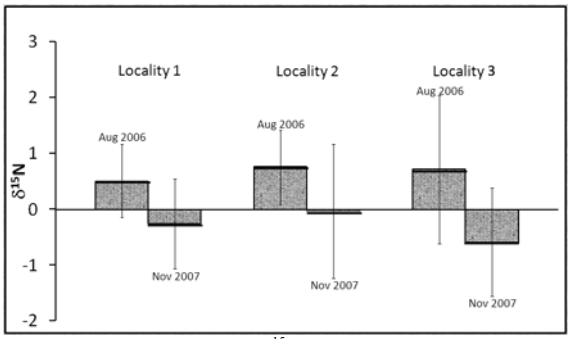

Figure 4. Average values of $\delta^{15}N$ differ seasonally between coral tissue and zooxanthellae at each of the three localities. During the dry season, the $\delta^{15}N$ values of the coral were heavier than those of the zooxanthellae, as indicated by the positive values, while during the rainy season, coral $\delta^{15}N$ values were lighter than zooxanthellae values.

Another possibility is that corals mainly consume zooplankton during the rainy season. However, during the dry season, the $\delta^{15}N$ value of the zooplankton was 7.1±1.2‰, while during the rainy season it was 5.1±0.1‰. These results show that the $\delta^{15}N$ values of marine zooplankton during both seasons were heavier than coral values. The heavier $\delta^{15}N$ values of the zooplankton imply that these branching coral reefs do not depend on zooplankton as their main food source.

Conclusion

This study documents the $\delta^{13}C$ and $\delta^{15}N$ values from three branching coral species; *Stylophora sp., Seriotopora sp.,* and *Porites sp.,* collected in two different seasons and at different turbidity levels. Different trends for the $\delta^{15}N$ values of coral tissue and the zooxanthellae were detected for each season. The majority of the coral tissue samples collected during the dry season had $\delta^{15}N$ values lighter than the zooxanthellae, but during the rainy season the opposite trend was observed.

Sedimentation rates only slightly affected the $\delta^{15}N$ values at the three localities. In spite of the fact that the coral polyps depend on zooxanthellae as a source of nutrients, our results suggest that the polyps also feed on POM from the surrounding waters which is probably produced during algal blooms (Muscatine and Kaplan, 1994). Moreover, coral tissues have previously been reported to have adapted their feeding habits from autotrophic to heterotrophic (Anthony and Fabricius, 2000). They are able to use POM as a food source.

Through direct observation and based on the results Suhendra (2006), we recognized that there is a difference in turbidity levels between Locality 1 and Locality 3. The sediment fraction at Locality 1 was dominated by silt which may come from the Berau River. The sedimentation rate for Locality 1 was also higher than the other localities (Suhendra 2006). However, this study did not detect isotopic differences between coral tissue and zooxanthellae in turbid water versus clear water.

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Reef-building coral *Goniastrea aspera* harbor a novel filamentous cyanobacterium in their skeleton

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Abstract. Reef-building corals harbor diverse communities of cyanobacteria, algae, bacteria and fungi within their skeleton. In spite of their potential significance, the interaction between these microbes and host corals is still obscure. Here we report a novel cyanobacterium within the skeleton of *Goniastrea aspera*, a massive reef-building coral that is predominantly found in shallow reef habitats. Characteristics of this cyanobacterium are: (1) non-branching filaments having a 1 μm diameter, (2) lack of heterocysts, (3) hormogonia formation, and (4) Chl *a* as the sole chlorophyll pigment. Consistent with these phenotypic traits, 16S rDNA sequence analysis showed a close association with this cyanobacterium to *Halomicronema,* a recently identified genus that includes species found in benthic microbial mats of hypersaline ponds. A possible interaction between *Halomicronema* sp. and the host coral is discussed in terms of stress tolerance.

Key words: Cyanobacteria, coral skeleton, endolithic algae, *Goniastrea aspera*, *Halomicronema* sp.

Introduction

The obligate symbiotic relationship between reef-building corals and dinoflagellates (zooxanthellae) is well recognized (D'Elia and Wiebe 1990). In addition to this well-established relationship, coral tissue and coral skeleton are also known to harbor diverse microbial consortia (Rohwer et al. 2002; Le Campion-Alsumard et al. 1995a). Until recently, however, these coral-microbial interactions remain obscure (Le Campion-Alsumard et al. 1995b)

The presence of endolithic microorganisms in skeleton of corals was first discovered in 1902 by Durden (Durden 1902). Visible green bands usually observed in the skeletons of massive corals comprised of cyanobacteria, fungi, bacteria, red and green algae (Le Campion-Alsumard et al 1995a; Schlichter et al. 1997). Among endolithic microbes, *Ostreobium* spp. are frequently found in coral skeletons (Lukas 1974).

The function of bioerosion that contributes to the geochemical and sedimentological importance in the reef was documented (Kobluk and Risk 1977). Also, endolithic microorganisms have been considered to be one of the major primary producers in coral reef environments (Tribollet et al. 2006). Recent findings have shown that endolithic algae transfer photoassimilates to coral host (Fine and Loya 2002; Lesser et al. 2007). This serves an alternative nutrient source especially during bleaching events. More recently, endolithic algae was reported to possess a photoprotective role in the coral-algal photosynthesis during high-light stress (Yamazaki et al. 2008).

Coral skeleton covered by living coral tissue is a harsh environment for the growth of many organisms (Shashar and Stambler 1992). This environment partly shares a similarity with that for microbial mats that harbor diverse microbial communities (Fourçans et al. 2006). In the intertidal reefs in Okinawa, Japan, massive coral *Goniastrea aspera* is constantly being exposed to a strong sunlight and high salinity during low tides. In this study, we report an endolithic community found within the skeleton of *G. aspera* .

Material and Methods

Colony of massive coral *Goniastrea aspera* (approximately 5-6 cm in diameter) was collected in June 2004 from a shallow intertidal pool of Bisezaki, Okinawa Japan. Coral tissue was removed using a WaterPik (Johhannes and Wiebe 1970) and coral skeleton was crushed to small fragments (approximately 1 cm in diameter) with a chisel. Skeletal fragments were then trimmed off with anatomical scissors to small pieces < 2 mm in length). The crushed skeletal pieces of *G. aspera* containing green bands were investigated utilizing culture method, microscopic observations, pigments analysis and molecular techniques.

Small pieces of coral skeleton were incubated in a liquid A medium (Mitsui and Cao 1988). Nitrogenous compounds were omitted from this medium to prevent the growth of undesirable microorganisms. The culture medium was modified to include sterile coral tissue extraction. Repeated subcultures of

colonies were carried out on agar plates to obtain a pure culture of cyanobacterium. All cultures were incubated at 28°C under a 12h dark : 12h light (30 µmol photons m^{-2} s^{-1}) photoperiod. The cultured cyanobacterium was used for pigment analysis and DNA analysis.

For microscopic observation, pieces of trimmed coral skeleton were ground in sterilized seawater using mortar and pestle. The samples were observed under a fluorescence microscope.

Pigments extracted from the green band and the cultured cyanobacterium were analyzed by thin layer chromatography analysis (TLC). Coral tissue was removed with a WaterPik as the described above. The tussue-removed skeletons were crushed in 90% acetone at 4°C, and were centrifuged at 10,000 x *g* for 2 min and the supernatant was used for TLC analysis. The cultured cyanobacterium was collected by centrifugation (15,000 x *g*, 3 min). The supernatant was then discarded and the cyanobacterial pellet was homogenized in 90% acetone at 4°C, and it was centrifuged at 15,000 x *g* for 3 min and the supernatant was used for TLC analysis. All procedures were carried out under a dim light. TLC analysis was carried out on a reverse phase C_{18} plate (MERCK) with 100% MeOH as the developer. The spots of chlorophyll pigments were visualized under a blacklight (UVP UVL-56).

Total genomic DNA of the cultured cyanobacterium was extracted using the UltraClean Soil DNA Kit (MoBio, Solana Beach, CA). PCR was performed using the primers of fD1 (5'-AGAGGATGATCAGCCACACTG-3') and rP2, which were designed for eubacterial 16S rDNA (Weisburg et al. 1991). The reaction mixture (50 µl) contained 0.15 mM deoxynucleotides (Takara, Tokyo, Japan), 0.2 µM forward primer, 0.2 µM reverse primer, 2µl of the PCR template and 0.05 U of recombinant *Taq* DNA polymerase (Takara) per µl in PCR buffer (Takara). The temperature program for 30 cycles of PCR was 94°C for 30 s, 55°C for 1 min, 72°C for 2 min, and 72°C for 5 min as the final extension after the last cycle. The amplified DNA fragment were purified by gel percolation. The sequence was determined in opposite orientations using a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with a DNA Sequencer (ABI 3100 Avant; Applied Biosystems). The obtained nucleotide sequence has been deposited in DNA Data Bank of Japan (DDBJ) with an accession number AB257773. Related sequences were aligned with CLUSTAL X 1.83 software for multiple sequence alignment, and bases of ambiguous alignment were corrected or removed manually. Tree topology was constructed with the neighbor joining method with MEGA 3.0 software (Kumar et al. 2004).

Results

Figure 1A shows the localization of green bands observed in the transverse section of *G. aspera*. It exhibits two distinct layers of green band within the skeleton. Green bands were observed just beneath and in deep of the skeleton that were separated by a light green or whitish zone. The green bands were primarily composed of photosynthetic organisms having non-branching, narrow filament approximately 1 µm in diameter as shown in Fig 1B and C.

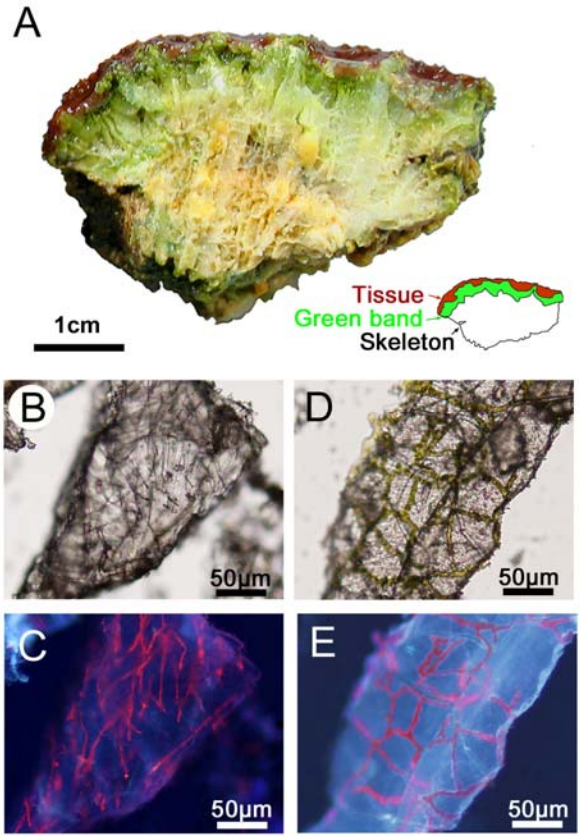

Figure 1: Layer of green band observed within the skeleton of *Goniastrea aspera*. (A) Dissected transverse section of *G. aspera* skeleton, (B and C) Photosynthetic organism found in skeletal fragments, (D and E) *Ostreobium* sp. (C) and (E) are epifluorescence images of (B) and (D), respectively.

Ostreobium sp. was observed on the fragments of the coral skeletons. The green alga showed repeatedly branching, non-septating 3 µm diameter filaments having knobby surface irregularities (Fig 1D and E). In addition to *Ostreobium* sp., coccoid photosynthetic organisms with less than 1 µm in diameter were also observed in the suspension of the green band and on the surface of skeletal fragments. These could be

attributed to the deposits during the process of tissue removal (data not shown).

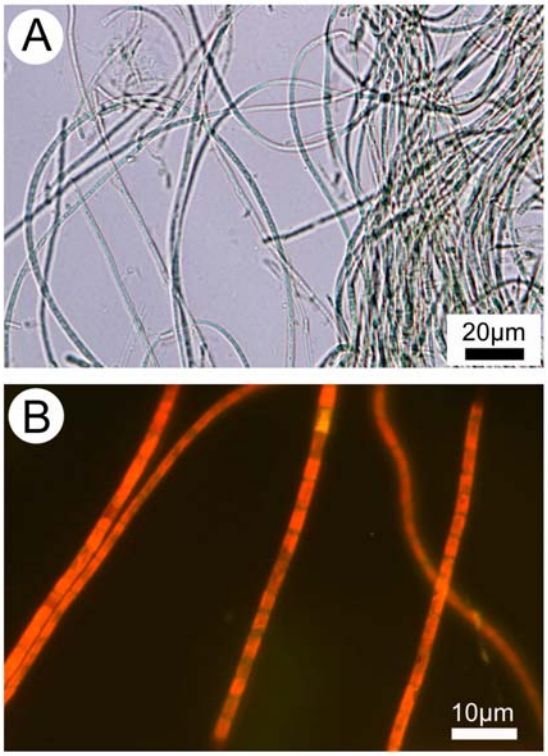

Figure 2: Morphology of the cultured filamentous cyanobacterium isolated from the green band in *G. aspera* skeleton. (A) Light micrograph of the filamentous cyanobacterium (B) Epifluorescence image of the filamentous cyanobacterium.

A cyanobacterium was successfully isolated and cultured with the modified A medium. This cultured cyanobacterium was non-branching, non-heterocyst forming, had narrow filaments around 1 μm in diameter and formed motile hormogonia (Fig 2). All these characteristics can be found in cyanobacteria belonging to the order Oscillatolariales.

Analysis of chlorophyll pigments using TLC revealed that both green band from coral skeleton and the isolated cyanobactrium contained chlorophyll *a* (Fig 3). However, chlorophyll *b* was only found in the extract obtained from the green band in the coral skeleton (Fig 3, lane A). Similar results were obtained with HPLC analysis (data not shown).

A phylogenetic tree constructed by the neighbor joining method is presented in Fig 4. A BLAST homology search of the determined sequence showed the closest identity (94%) to a 16S rDNA region of *Halomicronema excentricum*.

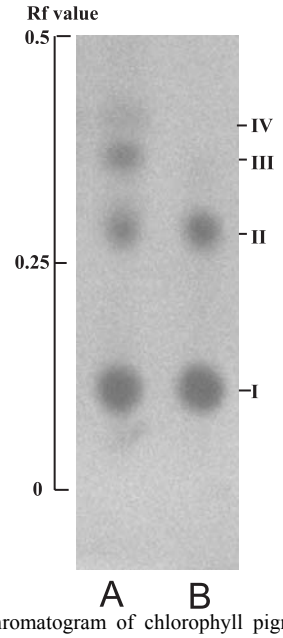

Figure 3: TLC chromatogram of chlorophyll pigments. Lane A, pigments extracted from the green band found in *G. aspera* skeleton. Lane B, pigments extracted from the isolated filamentous cyanobacterium. (I) Pheophytin *a* (II) Chlorophyll *a* (III) Pheophorbide *a* (IV) Chlorophyll *b*.

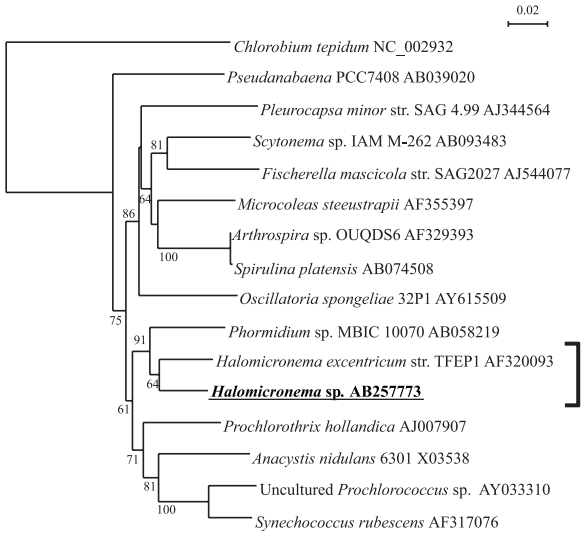

Figure 4: A phylogenetic tree constructed by the neighbor-joining method on the basis of 1029 bases 16S rDNA partial sequence. The sequence of *Chlorobium tepidum* was used as an outgroup. The scale bar represents a 0.05 substitution per nucleotide position. Numbers indicate the percentage of bootstrap support out of 1000 resampling data from the neighbor-joining method.

Discussion

To the best of our knowledge, this is the first report of a *Halomicronema* sp. inhabiting in the skeleton of a live coral. Species of the genus *Halomicronema* are one of the dominant cyanobacateria found in hypersaline microbial mat. They are characterized moderately halophilic and thermophilic (Abed et al. 2002; Fourçans et al. 2006). The present study

suggests that the internal environments of coral skeletons could be similar to the habitats for hypersaline microbial mats.

Shallow intertidal pools in coral reefs are harsh environments that accompany daily and seasonal changes in salinity and water temperature with large extents. The massive coral *G. aspera* can be dominantly found in such harsh habitats. It is interesting to note that even after the mass bleaching event in 1998, many *G. aspera* survived and increased in abundance around Okinawa Island (Loya et al. 2001). It appears that *G. aspera* is relatively stress tolerant.

Although further studies are needed to clarify the biological functions of the endolithtic microbes in *G. aspera*, we consider it plausible that the colonization brings a positive effect on the coral host. In fact, under high-light stress conditions, the *Acropora digitifera* colonies infected by endolithic microbes exhibited a photosynthetic activity higher than that of uninfected ones, a result suggesting a positive contribution of the microbes to the host physiology (Yamazaki et al. 2008). Endolithic microbes within coral skeleton may act as "secondary or facultative symbionts" (Yamazaki et al. 2008).

Endolithic algae found in coral skeletons have been reported to facilitate the host recovery from a bleached condition (Fine et al. 2002). *Halomicronema* was reported to have halophilic and thermophilic characteristics. High temperature and high salinity conditions in a shallow intertidal pool may allow the dominance of *Halomicronema* sp. within *G. aspera* skeleton. The presence of such stress-resistant microbes would contribute to the overall stress tolerance of *G. aspera*.

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From bacterial bleaching to the hologenome theory of evolution

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Abstract. Extensive bleaching of the coral *Oculina patagonica* in the eastern Mediterranean Sea occurs every summer when the seawater temperature exceeds 25°C. The infection by *Vibrio shiloi* and subsequent bleaching occurred only above 25°C because several of the bacterial virulence factors, superoxide dismutase, adhesion and toxin P, are only expressed above 25°C. Starting in 2002, the corals developed resistance to *V. shiloi*. Attempts to explain the resistance led to the Coral Probiotic Hypothesis, which posits that corals can adapt to their environment, including resistance to pathogens, by changing their symbiotic bacteria. The hologenome theory considers the holobiont a unit of natural selection. The hologenome is defined as the sum of the genetic information of the host and its microbiota. The theory is based on four well documented generalizations: (1) All animals and plants establish symbiotic relationships with microorganisms. (2) Symbiotic microorganisms are transmitted between generations. (3) The association between host and symbiont affects the fitness of the holobiont. (4) Under environmental stress, the symbiotic microbial community can change rapidly. These points taken together suggest that the genetic wealth of diverse microbial symbionts can play an important role both in adaptation and in evolution of higher organisms.

Keywords: Coral bleaching; *Vibrio*; holobiont, hologenome, evolution

Introduction

Extensive bleaching of the coral *Oculina patagonica* in the eastern Mediterranean Sea occurs every summer. Kushmaro et al. (1996, 1997) reported that the bleaching of *O. patagonica* was the result of an infection by *Vibrio shiloi*. The demonstration that *V. shiloi* was the causative agent of the disease was established by rigorously satisfying all of Koch's postulates, including the fact that bleached corals in the sea contained the bacterium (Kushmaro et al. 1996, 1997) whereas it was absent from healthy corals. Furthermore, Kushmaro et al. (1998) showed that the infection and subsequent bleaching only occurred at temperatures above 25°C. Thus, for bleaching to occur, both elevated temperature and the causative agent must be present.

The specific steps in the infection of *O. patagonica* by *V. shiloi* have been studied extensively (Rosenberg and Falkovitz 2004). The bacteria are chemotactic to the coral mucus, adhere to a β-galactoside-containing receptor on the coral surface, penetrate into the epidermal layer and multiply intracellularly, reaching 10^8 -10^9 cells per cm^3. The intracellular *V. shiloi* produces an extracellular peptide toxin (PYPVYPPPVVP) that inhibits algal photosynthesis. Another important factor for the virulence of *V. shiloi* is the expression of superoxide dismutase (SOD). Adhesion, production of the toxin and expression of SOD are all temperature-dependent reactions, occurring at summer (25-30°C) but not winter (16-20°C) temperatures. Thus, *V. shiloi* can not infect, multiply or survive in the coral during the winter. Sussman et al. (2003) demonstrated that the marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for *V. shiloi*.

Development of resistance of O. patagonica to V. shiloi

We have been studying the *V. shiloi/O. patagonica* model system of coral bleaching in the sea and in the laboratory for over ten years. Sometime between 2002 and 2004 we found that the corals became resistant to the pathogen. The evidence for the development of resistance is based on the following (Reshef et al. 2006):

1. From 1995-2002, the pathogen *V. shiloi* was readily isolated from 46/50 bleached and bleaching corals collected from the wild; from 2004 to the present, we have been unable to isolate *V. shiloi* from bleached or bleaching corals.
2. From 1995-2002, all laboratory strains of *V. shiloi* caused bleaching in controlled aquaria experiments; from 2004 to the present, none of the same strains bleach *O. patagonica* in the laboratory.
3. From 1995-2002, *V. shiloi* adhered to the corals, penetrated into the ectoderm and multiplied intracellularly to 10^8-10^9 cells per cm^3; now, *V. shiloi* adheres, penetrates the ectoderm and is rapidly killed.

Before the corals became resistant, the isolation and infection was so reproducible that for several years we used the system to demonstrate Koch's postulates in the teaching laboratory. Not only can we now **not**

isolate *V. shiloi* from bleached corals, but molecular techniques failed to recover the 16S rRNA gene from ca. 1000 clones that were sequenced (Koren and Rosenberg 2006). Recently, Ainsworth et al. (2008) confirmed that *V. shiloi* is not currently present in bleached *O. patagonica*, using FISH technology.

The coral probiotic hypothesis

The studies summarized above indicate that corals can indeed adapt rapidly to changing environmental conditions by altering their population of symbiotic bacteria. These studies led us to propose the Coral Probiotic Hypothesis (Reshef et al. 2006). This hypothesis posits that a dynamic relationship exists between symbiotic microorganisms and environmental conditions which brings about the selection of the most advantageous coral holobiont. Changing their microbial partners would allow the corals to adapt to changing environmental conditions more rapidly (days to weeks) than via mutation and selection (many years). An important outcome of the Probiotic Hypothesis would be development of resistance of the coral holobiont to diseases. The following evidence supports this hypothesis: (i) Corals contain a large and diverse bacterial population associated with their mucus and tissues. (ii) The coral-associated bacterial population undergoes a rapid change when environmental conditions are altered. (iii) Although lacking an adaptive immune system (no antibodies), corals can develop resistance to pathogens. One potentially contributing factor is the production of antibacterials by coral mucus bacteria (Ritchie 2006). The Coral Probiotic Hypothesis may help explain the evolutionary success of corals and moderate the predictions of their demise.

The hologenome theory of evolution: role of microorganisms in the evolution of animals and plants

The hologenome theory of evolution (Rosenberg et al. 2007; Zilber-Rosenberg and Rosenberg 2008) is a generalization of the coral probiotic hypothesis. More than a hundred years of biological research has demonstrated the importance of microorganisms in the health and disease of higher organisms. As a result of the recent development of culture-free molecular techniques, it is now accepted that in many cases the number of symbiotic microorganisms and their combined genetic information far exceed that of their hosts. The hologenome is defined as the sum of the genetic information of the host and its microbiota. In the hologenome theory of evolution, we suggest that the holobiont (Margulis 1993; Rohwer et al. 2002) (the host and its symbiotic microbiota) with its hologenome, should be considered a unit of selection in evolution, and that relatively rapid variation in the diverse microbial symbionts can have an important role in the adaptation and evolution of the holobiont. In essence, the hologenome theory of evolution focuses on the holobiont as a single dynamic entity in which a vast amount of the genetic information and variability is contributed by the microorganisms. Evolution of the holobiont can occur by changes in the host genome and/or in any of the associated microbial genomes, and relies on cooperation between the genomes within the holobiont, as much as on competition with other holobionts. A large body of empirical data provides the foundation for the hologenome theory of evolution. We choose to discuss this information within the following framework: (1) all animals and plants establish symbiotic relationships with diverse microorganisms. (2) Symbiotic microorganisms can be transmitted between generations with fidelity. (3) The association between host organism and its microbial community affects the fitness of the holobiont within its environment. (4) Genetic variation in holobionts can be enhanced by incorporating different symbiont populations and can change under environmental demand more rapidly and by more processes than the genetic information encoded by the host organism alone.

All animals and plants establish symbiotic relationships with microorganisms

Eukaryotes presumably arose from prokaryotes (Margulis 1993) and have remained in close relationship with them ever since. It is therefore not surprising that the surfaces of animals and plants contain a great abundance and variety of microorganisms. In addition, some microorganisms are able to grow inside animal or plant cells, i.e., endosymbionts. Because the vast majority of microorganisms that have been observed on or in animal and plant tissues cannot be cultured, current research on the diversity of microorganisms associated with a particular species relies primarily on culture-free DNA-based technology (Hugenholtz et al., 1998). Although censuses of microorganisms associated with different animal and plant species are only in an early stage, certain interesting generalizations have emerged: (1) The diversity of microbial species associated with a particular animal or plant species is high. (2) The host associated microbial community is very different from the community in the surrounding environment (Frias-Lopez et al. 2002; Rohwer et al. 2002; Sharp et al. 2007). (3) In some cases it has been shown that similar, but not identical, microbial populations are found on the same species that are geographically separated, while different populations are found on different species at the same location (Rohwer et al. 2002; Lambais et al. 2006; Fraune and Bosch 2007). (4) Different microbial communities often dominate different tissues of the same organism (Tannock 1995; Koren and Rosenberg 2006; Dethlefsen et al.

2007). (5) In several cases where a large diversity of associated bacterial species exists, certain bacterial groups dominate.

Transmission of symbionts between holobiont generations

The hologenome theory of evolution relies on ensuring the continuity of partnerships between holobiont generations. Accordingly, both host and symbiont genomes must be transmitted with accuracy from one generation to the next. The precise modes of vertical transmission of host genomes are well understood and need not be discussed here. However, in recent years, it has become clear that microbial symbionts can also be transmitted from parent to offspring hosts by a variety of methods. McFall-Ngai (2002), in an insightful review on the influence of bacteria on animal development, divided the modes for maintaining symbionts faithfully between generations into two categories, transovarian and environmental transmissions, while correctly acknowledging that there are numerous intermediate cases. We would like to take this approach one step further by suggesting that the numerous intermediate cases, in fact, best describe the large variety in modes of transmission, which are known at present to reconstitute plant and animal holobionts. It is this continuum of modes of transmission from direct to indirect that makes it impractical to place them in any specific categories.

Direct transmission from parent to offspring occurs with some symbionts where the microorganisms are in or on the reproductive cells. For example, in the aphid–Buchnera symbiosis, bacteria are intracellularly situated in bacteriocytes and are transferred to and transmitted via the eggs (Baumann et al., 1995). Direct contact is another slightly less direct mode of transmission demonstrated in mammals in which many of the symbionts are derived during passage through the birth canal or subsequently by close physical contact with parent or family and community members.

Another slightly less direct mode of transmission is used in the termite hindgut–microbiota symbiosis where feces of adult termites (containing abundant microorganisms) are fed to newly hatched juveniles by workers in the colony (Abe et al. 2000).

Cooperation between the host and the microbiota contributes to the fitness of the holobiont

Natural selection is the central concept of Darwinian theory – the fittest survive and spread their advantageous traits through populations, but 'fittest' is not an absolute property because it varies with environmental influences. Considering the holobiont as a unit of selection in evolution, we argue that the cooperation between the normal microbiota and the host generally leads to improved fitness. In addition, the genetic diversity of the microbiota can extend the range of environments in which the holobiont can compete successfully.

In several well-studied cases, neither the host nor the primary symbiont can survive without the other (absolute mutualism). For example, in the aphid–Buchnera symbiosis, the primary endosymbiotic bacterium has lost many genes required for independent growth during evolution, whereas the aphid partner depends on essential amino acids lacking in its diet that are synthesized and furnished by the symbiont (Baumann et al., 1995). While the aphid–Buchnera primary endosymbiosis is an example of absolute dependency, most of the symbiosis systems are not based on life or death interactions, but rather the microbial partners contribute in different degrees to the holobiont's well-being. One such example, also found in the aphids, is the secondary endosymbioses with bacteria belonging to a number of distinct lineages within the Gamma- and Alphaproteobacteria. These microorganisms are intracellular endosymbionts, which are not essential for growth and reproduction, but that contribute to the fitness of the holobiont (Russell et al. 2003). Regardless of the extent of dependency, a large body of data has been accumulated in recent years demonstrating that both endosymbionts and exosymbionts play numerous roles in metabolism, regulation, disease resistance and in sex and fertility determination (which may lead to species determination) of their hosts.

In the human gut–microbiota relationship a substantial amount of recent information has been gathered regarding many facets of this interaction (Xu and Gordon 2003; O'Hara and Shanahan 2006; Xu et al. 2007). The diverse interactions reported between the human gut and its microbiota include not only the cooperation in food breakdown [e.g. fiber into short-chain fatty acids (Hooper et al. 2002)] and biotransformation of certain molecules [e.g. bile acids (Hylemon and Harder, 1998)] but also participation in the development and normal function of the innate and adaptive immune systems in the gut (Hooper and Gordon 2001; O'Hara and Shanahan 2006), participation in the structural buildup of blood vessels [angiogenesis (Stappenbeck et al. 2002)] and participation in the regulation of fat accumulation (Bäckhed et al. 2004).

Genetic variation in holobionts

Variation is the raw material for evolution. According to the hologenome theory of evolution, genetic variation can arise from changes in either the host or the symbiotic microbiota genomes. Variation in host genomes occurs during sexual reproduction, chromosome rearrangements and ultimately by mutation. These same processes occur in microorganisms with the noteworthy difference that in haploid bacteria recombination occurs, within the same species, by conjugation, transduction and DNA transformation. In addition to recombination and

mutation, changes in the genome of the microbiota of holobionts can occur by three other processes: microbial amplification, acquisition of novel strains and horizontal gene transfer between different species. These three processes can occur rapidly under environmental demand and may be important elements in the evolution of animals and plants (Dinsdale et al. 2008).

Microbial amplification is the most rapid and easy to understand mode of variation in holobionts. It involves changes in the relative numbers of the diverse types of associated microorganisms that can occur as a result of changing temperatures (for plants and invertebrates), nutrient availability, exposure to antibiotics or other environmental factors. The holobiont is a dynamic entity with certain microorganisms multiplying and others decreasing in number as a function of local conditions. An increase in the number of a particular microbe is equivalent to gene amplification. Considering the large amount of genetic information encoded in the diverse microbial population of holobionts, microbial amplification is a powerful mechanism for adapting to changing conditions.

Another mechanism for introducing variation into holobionts is acquiring new symbionts from the environment. Animals and plants come in contact with billions of microorganisms during their lifetime. It is reasonable to assume that occasionally, as a random event, one of these microorganisms will find a niche and become established in the host. Under the appropriate conditions, the novel symbiont may become more abundant and affect the phenotype of the holobiont. Unlike microbial amplification, acquiring new symbionts can introduce entirely new genes into the holobiont.

Based solely on the host genome, animals and plants would evolve slowly because of (1) their relatively long generation times, (2) the fact that only changes in the DNA of the germ line are transmitted to the next generation and (3) often a whole set of new genes is required to introduce a beneficial phenotypic change. If the environment changes relatively rapidly, the host genome alone may not evolve quickly enough and the organism may lose competitiveness and become extinct. We argue that rapid changes in the symbiotic microbiota could allow the holobiont to adapt and survive under changing environmental conditions, thus providing the time necessary for the host genome to evolve. In some cases, the required new function remains within the symbiotic microbiota. For example, the animals that first evolved to feed on cellulose never developed the genes to degrade cellulose, but rather adapted to provide better conditions for microbial degradation of the polymer.

Discussion

The distinguishing feature of the hologenome theory is that it considers all of the diverse microbiota associated with the animal or the plant as part of the evolving holobiont and that changing the microbial community by amplification, novel acquisition from the environment and horizontal gene transfer provide additional mechanisms for rapid evolution. The consequences of considering all of the diverse symbiotic microbiota of the holobiont (endo and exocellular) are far reaching. Most importantly, they can increase the genetic information of the holobiont severalfold. Microbially directed processes can regulate and extend the metabolic capability of holobionts, including degradative and biosynthetic reactions in addition to regulatory mechanisms.

In terms of modern Darwinian theory, the unit of selection in evolution must contain two properties (Lloyd 1994): the replicator or genome that is subject to variation, and the interactor or phenotype, that interacts with the environment in a way that creates differential reproduction. The data reviewed in this article clearly demonstrate that both properties are expressed by the holobiont. The usefulness of considering the holobiont, with its hologenome, as a unit of selection is that it makes adaptability to changing environments in a relatively short time frame more comprehensible and amenable to experimental tests.

Interestingly, the hologenome theory incorporates aspects of both Darwinism and Lamarckism. Individual organisms evolve by selection of random variants, whereas the holobiont can evolve by adaptive processes. Consider, for example, marine invertebrates living in an ocean of increasing temperatures. The hologenome will change as a result of the shift-up in temperature by amplification of those microbiota symbionts that grow better at the higher temperature and possibly by gaining more thermophylic microorganisms from the environment. The holobiont will not only be better adapted to its environment, but it will also have an increased probability of passing on the acquired genetic traits to the next generation. Thus, when considering a holobiont, rather than the individual organisms that comprise it, it is possible to have an inheritance of acquired characteristics. How important this is in long-term evolution is debatable. However, in the short term, inheritance of acquired characteristics by holobionts may help them survive, multiply and buy the time necessary for the host genome to evolve.

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Dimethylsulfoniopropionate is linked to coral spawning, fish abundance and squid aggregations over a coral reef

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Coral reefs are frequently used as transient aggregation sites for foraging and spawning by marine fishes. These fishes may use predictable changes in chemical cues associated with reefs as signals to coordinate their aggregations. Dimethyl sulfide (DMS) and its precursor, dimethylsulfoniopropionate (DMSP), are ubiquitous compounds associated with productive areas, preferred sites for foraging and spawning of pelagic species. It is possible that species may recruit to DMS or DMSP (DMS/P) signatures associated with these areas. Here we investigate how temporal variation in the abundance of reef fishes and squid related to changes in DMS/P during a coral spawning event. During 2003, we recorded significant increases in water column DMS/P during a coral spawning event and found that an elevation in DMS/P was paralleled by a surge in numbers of pelagic fishes, *Caranx* spp*.,* and squid, *Loligo roperi,* over the reef. The changes in pelagic fish and squid abundance were positively correlated with DMS/P, suggesting that these animals may cue to the release of specific compounds during spawning. These results, coupled with other recent studies, provide further evidence that DMS/P may be used by fishes and squid to coordinate their aggregations over reefs.

Key Words: DMSP, pelagic fish, squid, aggregation, coral spawning

Introduction

Dimethyl sulfide (DMS) has been studied intensively by atmospheric and process-oriented oceanographers for its role in global climate, marine sulfur cycles and phytoplankton physiology (e.g., Curran and Jones 2000; Kiene *et al.* 2000). Dimethylsulfoniopropionate (DMSP) is a water-soluble osmolyte produced by species of marine phototrophic algae and is the major precursor of the volatile compound, dimethyl sulfide (DMS). DMSP is converted to DMS and acrylic acid by bacterial and algal enzymatic degradation (Kiene *et al.* 2000) and this metabolic conversion is accelerated during grazing by zooplankton (Dacey and Wakeham 1986; Daly and DiTullio 1996). Since the release of DMS and DMSP can be a by-product of zooplankton feeding (Cantin *et al.* 1996), its patchy distribution in the marine environment may reflect grazing rate (Kwint and Kramer 1996; Wolfe and Steinke 1996). Further, Hill and Dacey (2006) found that dissolved DMSP is released into the water immediately after foraging by planktivorous fishes.

Coral reefs are significant production sites for DMS and DMSP (Broadbent and Jones 2004). Reefs appear to produce DMSP in part through interactions between coral and their symbiotic zooxanthellae (Hill et al. 1995; Van Alstyne et al. 2006). Zooxanthellae taken from *Acropora* coral tissues from the Great Barrier Reef produce up to 285 fmol DMSP per cultured cell and up to 3831 fmol per cell in corals (Broadbent et al. 2002). Further, Broadbent (1997) reported an increase in water column DMS and DMSP the day after a mass coral spawning and attributed it to the corals' release of mucus and eggs containing zooxanthellae.

We are only beginning to understand the roles of DMSP and DMS as signal molecules in marine and terrestrial ecosystems (see Nevitt *et al.* 1995; Zimmer-Faust *et al.* 1996; Steinke *et al.* 2006; DeBose *et al*. 2006, 2007, 2008). High DMS and DMSP concentrations over productive marine areas can be long-lasting (hours to days; Ledyard and Dacey 1996) and thus, provide predictable cues that may be used by organisms to locate habitat. For instance, frontal zones are productive areas where seabirds (Nevitt 2000), Humboldt penguins (see Culik 2001), and basking sharks (Sims and Quayle 1998) forage on dense plankton patches. Nevitt (2000) has implicated air-borne DMS as part of an 'olfactory landscape', which can be detected by pelagic Procellariiform seabirds in search of productive areas for foraging. Humboldt penguins increase their anticipatory activity in the presence of DMS, suggesting that penguins could use DMS as a foraging cue as well (Culik 2001). Similarly, Sims and Quayle (1998) suggest that basking sharks might use DMS to locate dense patches of plankton along frontal zones.

There are many other examples of pelagic fish and other organisms that aggregate over particular sites in the marine environment. Many of these known aggregation sites are highly productive areas, such as frontal zones (Sims and Quayle 1998), seamounts (reviewed by Genin 2004), and coral reefs (reviewed by Domeier and Colin 1997). Could scented compounds associated with plankton-rich frontal zones and coral reefs, including DMS and DMSP, represent an aquatic counterpart to the olfactory landscape used by foraging seabirds?

Whether marine organisms use variations in DMS/P as habitat cues has never been explicitly tested, but there is evidence that they can detect DMSP at biologically appropriate concentrations. Nakajima and coworkers (1989, 1996) have shown through electrophysiological and behavioral trials that several species of fresh- and saltwater fish respond to DMSP (see DeBose et al. 2006). Initial field studies have also suggested that fish may be able to use DMSP as an aggregation cue in their natural environments (DeBose and Nevitt 2007; DeBose et al. 2008). Predictable temporal or spatial DMS/P signatures, such as those produced during coral spawning, might thus provide an 'olfactory landscape' which pelagic animals may use to locate particular habitat (see Nevitt 2000).

Here we explore whether fish and squid aggregations are correlated to fluctuations of DMS/P in a natural coral reef system. We investigated this idea by quantifying whether DMS/P levels over an offshore coral reef change during a coral spawning event and examining whether these changes in DMS/P are associated with temporal changes in the abundance of pelagic fishes and squid over the same period.

Materials and Methods

Study site

The Flower Garden Banks (FGB) consist of two upraised salt domes in the northwestern Gulf of Mexico, approximately 200 km off the Texas and Louisiana coasts (USA). The reef crests of the East and West banks peak at 22-28 m and 21-27 m depths, respectively, and are topped by a thriving coral reef with over 50% coral cover (Rezak et al. 1985).

Total DMS and DMSP measurements

Water collection

Seawater samples were collected in Corning 50 mL plastic centrifuge tubes. Samples were taken by scuba divers, along 100 m transects at approximately 12 m depth (mid-water above the coral reef). Results of a preliminary study showed mid-water collections to represent the highest concentrations of total DMS+DMSP (DMS/P) (see DeBose 2008). Samples for total DMS/P were collected using scuba on August 4, 5, 18-21, 2003. Six samples were taken per dive and samples were collected on August 4-5 during the morning between the hours of 06:54-07:18 and 10:58-11:24 local time (Central Standard Time). On August 18-21 both day and night samples were taken for comparison between the hours of 16:30-17:18 and 21:10-21:35. The timing of night sampling was based on a historical record of coral spawning events and estimated from the previous years' spawning times (Gittings et al. 1992; Vize et al. 2005). Sample times were consistent over nights, so even though samples may not have been collected during peak spawning activity, the samples reflect day to day changes in DMS/P. Samples were collected prior to the coral spawning on the night of August 18, so these samples were classified, along with samples collected on August 4-5, as within the "Pre-Spawn" period. Water samples collected on August 19-21 were classified as within the "Spawn" period. After surfacing from each dive, we pipetted 20 mL of the seawater samples into '20 mL' vials and added 1 mL of 1 N sodium hydroxide (NaOH). Vials were then sealed with Teflon-coated, butyl rubber septa and aluminum caps, crimped, and stored upside down in the dark.

Water chemistry analysis

Whole water samples were preserved with 1 N NaOH, which cleaves DMSP (particulate and dissolved) into DMS, so that the two sources of DMS could not be differentiated in this study. All concentrations are therefore reported as DMS/P.

Total DMS+DMSP was quantified using a gas chromatograph (GC; Shimadzu GC-14A equipped with a FPD-14) at the Dauphin Island Sea Lab, Alabama, USA. Samples were analyzed January 26-28, 2004. Our results can be considered conservative estimates of total DMS/P, since some loss of DMS from samples might have occurred during storage.

Each 20 mL sample was vortexed, and 1 mL of the sample was then combined with 1 mL of 5 N NaOH in a 5 mL serum vial. Headspace gas in the vial was captured using a modified cryotrapping method adapted from Kiene and Service (1991). The oven temperature was maintained at 70°C to adequately separate sulfur compounds. Standard preparation is described elsewhere (Kiene and Service 1991).

Coral Spawning Behavior

Seven to ten days after the first full moon in August, seven species of hermatypic corals of the Flower Garden Banks undergo their annual spawning (Gittings et al. 1992). Scuba dives to observe coral spawning were made from the diving vessel M/V Spree (Gulf Diving LLC) at the West Flower Garden Bank from August 18-20 and at the East Bank on

August 21. Observations were made by two teams of scuba divers (six total divers) for approximately 500.5 min per diver, at depths ranging between 18 and 30 m. Divers monitored coral spawning behavior every night between the hours of 20:30 and 24:00 from August 18-21. Each night, divers entered the water for consecutive, overlapping time periods in order to continuously monitor the onset and completion of coral spawning within 100 m radius of the boat. Divers quantified the number and species of coral heads spawning, and the times of spawning activity for each species.

Quantification of Fish and Squid Abundance

We used Reef Environmental Education Foundation (REEF) Roving Diver Technique surveys to quantify the abundance of reef fishes (see Pattengill-Semmens and Semmens 1998). REEF surveys were conducted between 07:00 and 22:00 and individual survey times lasted approximately 42 ± 8 min (n=72). Fish abundances were scored as: 0 (Absent), 1 (Single), 2 (Few, 2-10 individuals), 3 (Many, 11-100), 4 (Abundant, >100). Abundance scores were interpreted using a Density Index score: DI = {(S*1) + (F*2) + (M*3) + (A*4)} / (number of surveys in which species was observed) (REEF 2004).

Squid counts were recorded by instantaneous point sampling throughout the dive, using a remotely operated vehicle (ROV; Phantom, operated by National Undersea Research Center/UNCW) on August 5, and by scuba on August 4, 18-21. On August 4, dive time was 46 min (from 20:50 to 21:36). ROV dive time was 152 min (from 18:15 to 20:47). During the spawning week, scuba dives were made between the hours of 20:32-00:07. The observation time by any one dive team ranged from 443-586 minutes over four nights, with the average nightly dive lasting 110-146 min.

Statistical Analyses

A mixed, nested ANOVA was used to analyze the effects of several factors on water column DMS/P: Date, Period (Pre-Spawning or Coral Spawning), Bank (East or West Flower Garden Bank), and Day/ Night collection. Date was nested within Period and Bank. The final ANOVA model was obtained by backwards elimination (eliminating factors with F < 1). Fish DI scores and squid counts failed to conform to normality and could not be transformed. Therefore, DI scores and squid numbers were analyzed using non-parametric Spearman's Rho Correlation. All statistical tests were two-tailed and performed using Statistica (StatSoft, Inc., Tulsa, OK).

Results

Total DMS+DMSP

The August 2003 spawning event (Table 1) occurred within the predicted time period (see Vize *et al.* 2005). Associated with this period of coral spawning, we observed a significant increase in water column DMS/P (Fig. 1; $F_{1,55}$ = 23.17, P = 0.0172). DMS/P levels varied among days, and showed a significant increase beginning on the first day after the initial coral spawning ($F_{3,55}$ = 3.717, P = 0.0166). DMS/P did not vary between day and night samples ($F_{1,53}$ = 0.0127, P = 0.9106). Neither did DMS/P vary between the East and West Flower Garden Bank ($F_{1,55}$ = 1.125, P = 0.3695).

Species	18-Aug-03	19-Aug-03	20-Aug-03	21-Aug-03
Montastraea cavernosa Great star coral	2030–2138 (10)	--	--	--
Montastraea franksii Mountainous star coral	2140–2214 (50)	2212–2230 (20)	--	--
Diploria strigosa Symmetrical brain coral	2200 (2)	2130–2230 (15)	--	--
Stephanocoenia intersepta Blushing star coral	2250 (2)	2250–2257 (2)	--	--
Montastraea faveolata Mountainous star coral	--	--	2300–2330 (5)	
Colpophyllia natans Large-grooved brain coral	--	--	2040–2110 (3)	2045–2105 (17)

Table 1: Spawning times for each broadcast spawning coral species during August 2003. The number of coral heads observed spawning is in parentheses next to time frames detailing when the first and last coral head of each species released spawn.

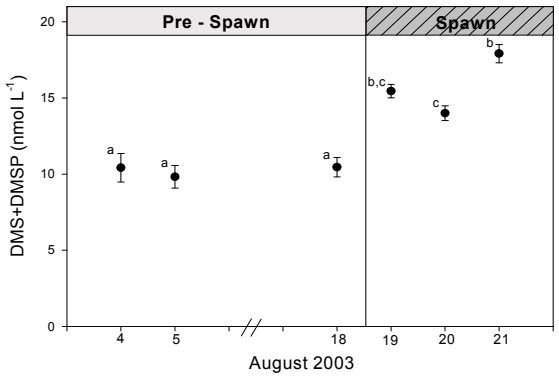

Figure 1: Total DMS+DMSP (nmol L^{-1}) in the water column increased significantly during coral spawning. Letters indicate a significant difference between days (see text).

Fish and Squid Abundance

Divers counted 133 fish species over the six days of sampling, with a maximum of 96 species on August 20-21. We found significant positive relationships between density scores for *Caranx hippos* (Fig. 2; $(r_s)_{0.05(2),6}$ = 0.8971, P = 0.0153) and *C. latus* ($(r_s)_{0.05(2),6}$ = 0.9095, P = 0.0119) and DMS/P. There was also a significant correlation between numbers of *Loligo roperi* ($(r_s)_{0.05(2),6}$ = 0.8971, P = 0.0153) and DMS/P. The abundances of *Caranx* fishes and

number of squid were not significantly related to the number of corals reported spawning (*C. hippos* $(r_s)_{0.05(2),6} = 0.6470$, $P = 0.1649$; *C. latus* $(r_s)_{0.05(2),6} = 0.7276$, $P = 0.1012$; *L. roperi* $(r_s)_{0.05(2),6} = 0.4706$, $P = 0.3462$).

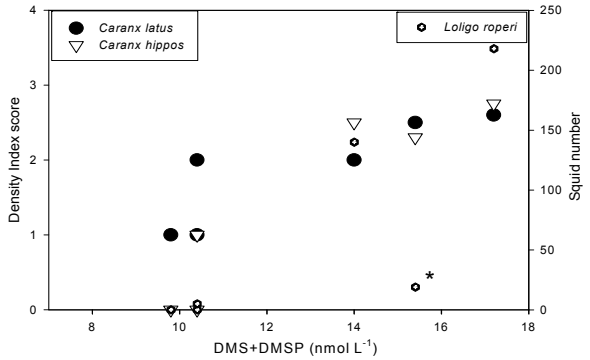

Figure 2: The density index scores for pelagic jacks (circles and triangles) and numbers of *Loligo roperi* (starred hexagons), showed positive correlations with temporal changes in DMS/P over the coral spawning period. DI scores and squid numbers are plotted against the average daily DMS/P. Asterisk denotes delay in squid numbers until after the first peak of DMS/P, which occurred on the first night of the coral spawning.

Discussion

Water column DMS/P increased significantly over the coral spawning period as compared to pre-spawning dates. The higher DMS/P concentrations were accompanied by increased abundances of pelagic fishes (*Caranx hippos* and *C. latus*) and squid (*Loligo roperi*) during the spawning period. These increases in fish and squid were associated with temporal variations in water column DMS/P rather than with the number of corals spawning.

Our data indicate that fish and squid are attracted to the coral spawning event, as evidenced by the increase in their abundances that we documented here (see also Westneat and Resing 1988; Pratchett et al. 2001). More specifically, we found the abundances of fish and squid were correlated with water column levels of DMS/P, suggesting that fish and squid may be using chemical DMS/P signatures as part of an olfactory landscape to time behaviors leading to aggregations. Based on this correlation, and similar behaviors elicited by an experimental release of DMSP (DeBose et al. 2008), we hypothesize that this attraction is mediated by temporally distinct elevations in DMS/P, or associated compounds in the water column, which serve as cues to help coordinate these behaviors. Additional chemical cues released either during spawning or by acts of predation, coupled with a changing visual landscape as fish recruit to the area, may draw in additional predatory, pelagic fishes. While in the present study we discuss this hypothesis over reef habitats, similar scenarios might also occur in upwelling areas, where primary productivity is elevated and zooplankton grazing on plankton releases substantial amounts of DMS/P and other chemicals (see Steinke et al. 2002).

Loligo roperi numbers were also significantly correlated with high levels of DMS/P. An apparent delay in the increase in squid number (shown in Figure 2) suggests squid might be using DMS/P to coordinate their activity on the reef. The schools of *Loligo roperi* recorded over the Flower Garden Banks consist of mated/mating adults and reproductively immature juveniles; the Flower Garden Banks are the only known mating grounds for this elusive squid (DeBose and Vecchione 2005).

The proximate causes for the increase in DMS/P are still undetermined. Zooxanthellae harbor high amounts of DMSP (73-117 fmol cell^{-1}: Hill *et al.* 1995; 48-285 fmol cell^{-1}: Broadbent *et al.* 2002), and the amount of zooxanthellae within coral spawn, including eggs and mucus (Muller-Parker and D'Elia 1997; Broadbent and Jones 2004) could produce a substantial release of DMSP. The highest levels of DMS and DMSP ever recorded in the marine environment were found within coral mucous ropes (DMS: 17.9 µM; DMSP: 44.9 µM) and surface waters (DMS: 219 nM; DMSPd: 630 nM) of the Great Barrier Reef (Broadbent and Jones 2004). Peak DMS/P concentrations over the FGB (2002-2003: 9.8 – 49.4 nM) did not reach the levels found by Broadbent and Jones (2004), however, the FGB are offshore reefs with notably different current regimes and tidal influences: corals do not breach the sea-surface, and mucous ropes do not appear to get trapped and aggregated within the area. Also, due to our whole-water sampling technique, the DMS/P totals could be considered averaged whereas odor plumes tend to have a filamentous structure containing high-concentration eddies (reviewed by DeBose and Nevitt 2008). This means that organisms perceiving DMS/P on finer scales would sense a higher concentration than we could capture in our samples (see Moore and Crimaldi 2004). Though the mechanism of DMSP release is still largely unknown, the evidence presented here suggests that coral spawning behavior of an off-shore reef is directly associated with increases in DMS/P over the reef.

In summary, we found an increase in water column DMS/P associated with a coral spawning event in the northwestern Gulf of Mexico. This increase was correlated with changes in pelagic fish and squid abundance. Our observations suggest that a natural increase in DMS/P might serve as a cue for foraging and/or spawning of pelagic fishes and squid. This idea provides a specific, testable hypothesis for examining how the sensory ecology of marine fishes and squid may be responsive to chemicals that originate from

the behaviors of other species (i.e., coral spawning, zooplankton foraging). Still, our explorations into this area are only beginning (see DeBose and Nevitt 2007; DeBose et al. 2008), and future work is needed to investigate DMS and DMSP as signal molecules in the marine environment.

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Are tropical herbivores more tolerant of chemically rich seaweeds than are temperate herbivores? A test of seaweed-herbivore coevolution

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Abstract. There is a greater quantity and diversity of lipophilic secondary metabolites produced by seaweeds within tropical relative to temperate regions. Coevolutionary arms-race theory predicts that tropical herbivores should more readily tolerate consuming chemically-rich seaweeds than do temperate herbivores, however, tests of this prediction are rare. We assessed the willingness to consume freeze-dried tissue from 10 species of chemically-rich seaweeds exhibited by multiple populations of the herbivorous amphipod *Ampithoe longimana* collected from cold-temperate, warm-temperate and subtropical estuaries along the east and Gulf coasts of North America. In contrast with the coevolutionary predictions, geographic patterns of consumption of chemically-rich seaweeds were not correlated with latitude. Rather, populations in North Carolina (34ºN) consumed more tissue from the diterpene-producing seaweed genus *Dictyota* than did New England (41ºN) or Florida (27ºN) populations. Amphipod populations did not differ in their feeding responses to tissue from chemically-rich seaweeds in the genera *Amphiroa, Halimeda, Padina, Penicillus,* nor *Udotea*. Thus, Floridean *A. longimana* have not locally evolved a feeding tolerance for chemical defenses produced by tropical seaweeds, as would be predicted by an arms-race hypothesis. Rather, the geographic patterns in feeding behaviors reflect the relative importance of these seaweeds to the ecology of local populations.

Keywords. Herbivore offense, seaweed chemical defense, coevolutionary arms-race, biogeography

Introduction

Marine herbivores face profound challenges when feeding on seaweeds. Seaweeds and vascular plants generally contain low levels of nitrogen relative to herbivore tissues (Horn 1989; Choat and Clements 1998), which require herbivores to consume large quantities of plant material to maintain themselves. In addition, seaweeds have evolved structures and morphologies (e.g., crusts and calcification) that can make the algae tougher and less profitable to consume (Littler and Littler 1980; Steneck 1986; Schupp and Paul 1994). Finally, seaweeds contain an arsenal of secondary metabolites, including terpenoids, aromatics, and acetogenins, which deter herbivores (Paul 1992) and require biochemical manipulation (Targett and Arnold 2001; Sotka and Whalen 2008).

There is emerging evidence that these feeding challenges can be more profound for tropical herbivores relative to their temperate counterparts. Specifically, the concentration and diversity of lipophilic secondary metabolites within tropical seaweeds are considerably higher than those within temperate seaweeds (Hay 1991; Paul 1992; Van Alstyne et al. 2001; Pereira and de Gama 2008). In one of the few direct tests of a tropical-temperate gradient in seaweed defenses, Bolser and Hay (1996) conducted feeding assays with two urchin species (*Lytechinus variegatus* and *Arbacia punctulata*) to demonstrate that freeze-dried tissue from temperate, North Carolina seaweeds were consumed at approximately twice the rates of tissue from closely-related tropical, Bahamian seaweeds. The authors also found that the higher palatability of temperate seaweeds was mediated by either the quality or quantity of secondary metabolites within the lipophilic extract. In contrast, variation in palatability among tropical and temperate seaweeds was not mediated by water-soluble compounds or the content of protein and ash-free organic matter.

There is general agreement that this greater deterrence of tropical seaweeds and their lipophilic metabolites relative to temperate seaweeds represents a macroevolutionary response by tropical seaweeds to an intensification of herbivory rates (Hay 1991; Paul et al. 2001; Van Alstyne et al. 2001). The greater herbivory pressures in the tropics are due in part to dramatically greater diversity and abundance of herbivorous fishes in the tropics relative to temperate regions (Gaines and Lubchenco 1982; Floeter et al. 2004). However, a neglected explanation of latitudinal trends in herbivory is the notion that tropical herbivores have a greater

feeding tolerance for chemically-defended tropical seaweeds than temperate herbivores. This geographic difference in feeding tolerance would evolve if tropical herbivores have responded to the greater levels of chemical defense of their prey via a diffuse evolutionary arms-race (cf. Vermeij 1994).

One of the few tests of an arms-race hypothesis for marine plant-herbivore interactions is Cronin et al. (1997). The authors found that diterpenoid metabolites from the tropical Pacific seaweed *Dictyota acutiloba* deterred North Carolina pinfish *Lagodon rhomboides* and the purple sea urchin *Arbacia punctulata* at concentrations that did not deter fish (*Scarus shlegeli*, *Scarus sordidus*, *Naso lituratus*, and *Naso unicornis*) and an urchin *Diadema savignyi* from tropical reefs of Guam. A second example comes from the combined results of Targett et al. (1995) and Boettcher and Targett (1993), which reveal that high concentrations of polyphenolics (~10% of tissue dry weight) lowered the assimilation efficiency (AE) of the temperate fish *Xiphaster mucosus*, but did not alter the AE of two tropical fishes (*Sparisoma radians* and *S. chrysopterum*) and a tropical crab (*Mithrax sculptus*). Because the temperate fish had a far more acidic gut (pH 2-3) relative to the tropical herbivores (pH 5-9), it was suggested that hydrogen bonding among polyphenolics and proteins is enhanced within acidic guts (Targett et al. 1995).

These studies tested the arms-race hypothesis using multiple herbivore species from temperate vs. tropical regions, but another approach is to compare geographically-isolated populations within a single herbivore species. Here, we assessed the relative feeding preferences for 10 species of chemically-rich seaweeds exhibited by the herbivorous amphipod *Ampithoe longimana* collected from cold-temperate (New England; 41ºN), warm-temperate (North Carolina; 34ºN) and subtropical (Florida; 27ºN) estuaries along the east coast of North America. The small (<1cm adults) gammaridean amphipod (Arthropoda; Crustacea; Malacostraca) is a tube-dwelling brooder that lives and feeds on a variety of algae (Duffy and Hay 1991; Sotka and Hay 2002; McCarty 2008). Our arms-race prediction is that subtropical populations of *A. longimana* should more readily consume tropical, chemically-rich seaweeds relative to amphipod populations from more temperate regions.

Materials and Methods

Specimen Collection and Storage

Ampithoe longimana specimens were collected at low tide from along 1,700 kilometers across the Atlantic coast of the United States in three regions: New England (Westport, Massachusetts (41º31'N, 71º04'W), Jamestown, Rhode Island (41º29'N, 71º23'W), and Niantic, Connecticut (41º18'N, 72º10'W)); North Carolina (Harker's Island (34º43'N,76º35'W), Morehead City (34º42'N,76º40'W), and Wrightsville Beach (34º13N, 77º48'W)); and Florida ((Tampa Bay 27º45'N, 82º37'W) and Fort Pierce (27º27'N, 80º19'W)) between May of 2006 and August of 2007. We are confident that these populations are indeed *A. longimana*: Connecticut, Massachussetts and North Carolina populations readily interbreed (Sotka 2003) and published data (Sotka et al. 2003; McCarty 2008) indicate that Florida and Connecticut populations differ from each other by ~2% at a mitochondrial locus.

Amphipods were collected by hand from a variety of seaweeds and transported to Grice Marine Laboratory. Amphipods were cultured in ~30 ppt filtered seawater obtained from Grice Cove in Charleston, South Carolina at 20ºC. Water was changed every 5-7 days, and the containers were aerated constantly. Amphipods in the cultures were fed fresh *Ulva* spp., *Ectocarpus* spp., *Sargassum* spp., *Bryopsis* spp., *Gracilaria* spp., and *Hypnea* spp. collected from throughout North and South Carolina. All seaweeds were rinsed at least twice in freshwater for 30-45 seconds to remove local biota prior to being placed in the cultures. Because amphipods used in our feeding assays represent at least the 2nd generation in these 'common garden' cultures, population-level patterns in feeding behaviors likely have a genetic basis.

Seaweeds used for the feeding assays were collected in May 2005 from the upper Florida Keys (25º07'N, 80º25'W; *Halimeda* incrassata, *H. opuntia*, *H. tuna, Penicillus dumentosus, Udotea flabellum*, an unidentified *Dictyota* sp. and an unidentified *Amphiroa* sp.) and in July 2005 from near Morehead City, NC (*Dictyota cilolata*, *D. menstrualis*, *Padina gymnospora*) and in February-March 2005 from Charleston Harbor, SC (34º45'N, 79º54'W; *Ulva (syn. Enteromorpha) intestinalis*). Seaweeds were subsequently frozen at -4°C within 12 hours of collection, lyophilized, ground to a powder, and stored at -20°C for long-term storage.

Feeding Assays

To assess feeding preferences, a series of choice assays were performed using lyophilized tissue. Offering lyophilized tissue permitted experiments at different times without changing tissue quality. Lyophilization removes morphological traits but largely maintains biochemical traits that might mediate feeding choices. The process of lyophilization reduces the quantity of some secondary metabolites (Cronin et al. 1995). However, it is unlikely this potential artifact biases our conclusions because we are primarily focused on the relative differences in feeding behavior between populations exposed to the same foods.

Frozen, lyophilized algae were ground to a fine powder using a Wiley mini-mill with a 180 mm mesh sieve or ground by hand using a mortar and pestle. Food was prepared using the following recipe: 0.5 g freeze-dried seaweed mixed with 2mL H_2O added to 0.9

g of agar that was mixed and heated with 2.5 mL H_2O. The recipe was doubled, tripled or quadrupled to make sufficient food for each assay. This agar and seaweed mixture was applied to plastic screen mesh (i.e. window screen) and pressed between two sheets of wax paper for ~ less than one hour. The mesh was then cut into feeding grids measuring 5 x 6 mesh squares.

A single replicate assay consisted of one 200 mL plastic cup with ~75 mL of seawater, and a single feeding grid each of the 'Control' (*Ulva (syn. Enteromorpha) intestinalis*) and 'Treatment' food (*D. menstrualis, D. ciliolata,* etc.). Each 'Treatment' seaweed species is tropical in distribution, and is a low-preference food for several generalist fish, urchin and amphipod consumers largely because of their low nutritional value, chemical deterrents, and/or morphological defenses (Paul 1992; Bolser and Hay 1996; Pereira and de Gama 2008). *U.intestinalis* was chosen as a 'Control' because it is locally available to temperate and tropical populations of the amphipod, is readily consumed by *A. longimana*, and there are no population differences in fitness when fed *U. intestinalis* (Sotka et al. 2003).

One amphipod was placed into each replicate with the two feeding grids. Replicates were placed in covered containers in either a 20ºC or 25ºC incubator in complete darkness for the duration of the assay. Replicates were checked twice daily and stopped when five days had lapsed, or when the amphipod had consumed either 9 squares from one feeding square or a combination of 10 squares from both feeding squares, whichever was first. Replicates with fewer than 9 squares, or more than 35 squares, were excluded from analysis. Most replicates were completed within 2-3 days, but this is sufficient time to allow soluble materials to leach, including proteins, amino acids, carbohydrates and any phlorotannins. Lipophilic compounds should, however, maintain themselves on the artificial foods.

Extremes in local temperatures alter herbivore feeding behavior (Sotka and Giddens 2009). Thus, because New England populations rarely experience 25ºC, Floridean populations rarely experience 20ºC, and North Carolina populations experience both temperatures annually (Sotka and Giddens 2009), we present assay results from New England amphipods at 20ºC and North Carolina and Florida populations at 25ºC.

Paired t-tests were used to assess whether consumption rates of the two foods differed (i.e., Control vs. Treatment). To assess whether populations differed in their feeding choices, we converted the data into a proportion of treatment tissue consumed (e.g., amount of Treatment consumed divided by total amount consumed in a replicate) and analyzed this proportion using a nonparametric ANOVA using a distribution generated from a permutation method (Anderson 2001). If we had data from multiple populations within a region, then we combined all replicates for that region for analysis.

Results

During feeding choice assays, cold-temperate (New England), warm-temperate (North Carolina) and subtropical (Florida) populations consumed significantly more *Ulva* than any of the green seaweeds (*Halimeda* incrassata, *H.* opuntia, *H. tuna, Penicillus dumentosus* and *Udotea flabellum*), the red seaweed *Amphiroa* and the brown unidentified *Dictyota* species (Paired t-test $p < 0.05$ for each population / seaweed combination; Table 1). In contrast, amphipod populations tended to consume statistically equivalent amounts of *Ulva* and the brown seaweeds *Padina gymnospora, D. ciliolata* and *D. menstrualis.* However, Florida amphipods ate significantly more *Ulva* than *D. ciliolata* while North Carolina amphipods ate significantly more *D. menstrualis* than *Ulva*.

Table 1. Relative consumption of 10 chemically-rich tropical seaweeds by three populations of the herbivorous amphipod *Ampithoe longimana*. The proportion of chemically-rich seaweed consumed, sample size (n) and *p*-values are indicated for each assay. Grey boxes highlight assays where the consumption of treatment foods was significantly different (i.e., less or more) than of control foods.

	Florida (27ºN)			North Carolina (34ºN)			New England (41ºN)		
Seaweed	Proportion	n	*P-value*	Proportion	n	*P-value*	Proportion	n	*P-value*
Amphiroa	0.24	31	<0.001	0.15	36	<0.001	0.07	33	<0.001
D. ciliolata	0.24	36	<0.001	0.51	38	0.203	0.43	33	0.364
D. menstrualis	0.44	40	0.521	0.64	29	0.018	0.42	15	0.578
Dictyota sp.	0.29	35	<0.001	0.35	36	0.038	0.21	37	<0.001
Halimeda incrassata	0.07	36	<0.001	0.02	21	<0.001	0.08	37	<0.001
H. opuntia	0.08	33	<0.001	0.07	9	<0.001	0.01	40	<0.001
H. tuna	0.11	32	<0.001	0.19	28	<0.001	0.16	22	<0.001
Padina	0.36	38	0.161	0.37	29	0.436	0.43	24	0.583
Penicillus	0.21	35	<0.001	0.26	35	<0.001	0.28	38	<0.001
Udotea	0.24	35	<0.001	0.18	27	<0.001	0.12	36	<0.001

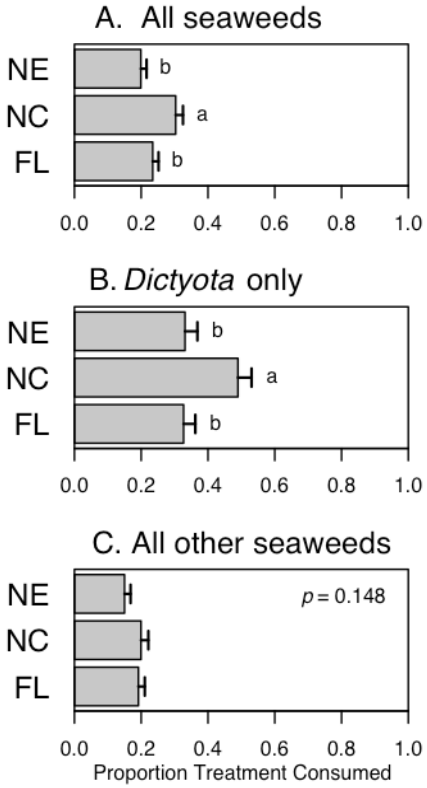

Figure 1. The average (+ S.E.) proportion of chemically-rich tropical seaweed consumed. Letters indicate groups that were significantly different.

We explicitly tested whether there are significant differences between populations in the proportion of chemically-rich tissue consumed. When all replicates are pooled, North Carolina individuals consumed significantly more of the chemically-rich tissue than did either Florida or New England populations (Figure 1A). However, as is suggested from Table 1, the regional differences in feeding preferences are driven largely by geographic variation in consumption of *Dictyota menstrualis and D. ciliolata*. Moreover, previous work has indicated that North Carolina animals use *D. menstrualis* and *D. ciliolata* as a host (Duffy and Hay 1991; Sotka and Hay 2002; Sotka et al. 2003). When we re-analyzed the feeding responses of the amphipods toward the genus *Dictyota* vs. the feeding response to the remaining seaweeds, North Carolina individuals consumed significantly more *Dictyota* tissue than did New England or Florida individuals (Figure 1B). When *Dictyota* seaweeds are removed from analysis, then there is no significant difference between the populations in the propensity to consume the other species of chemically-rich seaweeds (Figure 1C).

Discussion

Our results indicate that subtropical amphipod populations do not exhibit greater feeding preference for tropical chemically-rich seaweeds than do more temperate amphipods (Table 1; Figure 1). Rather, the warm temperate North Carolina amphipods were more likely than either the cold temperate New England or Florida populations to consume species in the genus *Dictyota*. This result does not support the notion that Florida amphipods have responded evolutionarily to chemically-rich tropical seaweeds, as would be predicted by an arms-race hypothesis.

Instead, local host use is central to understanding the evolution of these feeding behaviors (McCarty 2008). North Carolina amphipods feed readily on *Dictyota* and are readily found on *Dictyota* (Duffy and Hay 1991; McCarty 2008). This is because the *Dictyota* produces diterpene alcohols that deter larger omnivorous pinfishes that dominate the local benthos. By associating with this chemically-defended seaweed, the amphipods gain a measure of protection from their fish consumers. Neither New England nor Florida amphipods are found on *Dicytota* because *Dictyota* is not available in their local habitats (McCarty 2008). New England populations are 100's of kilometers north of the northern endpoint of *Dictyota*. In Florida estuaries, *A. longimana* are abundant where *Dictyota* appears to be rare, while on Florida coral reefs, *Dictyota* is abundant while *A. longimana* have never been found. Thus, the greater preference for *Dictyota* seen among North Carolina individuals reflects the importance of this seaweed in its local ecology.

Similarly, the lack of a regional response in feeding behavior toward the other chemically-rich seaweeds (e.g., *Amphiroa*, *Udotea*, etc.) reflects the fact that the amphipod is rarely found naturally on these seaweeds (McCarty 2008). The Florida *A. longimana* are found within estuarine seagrass beds where chemically-rich seaweeds are rare and where there are abundant seaweeds that lack chemical, morphological or mineral defenses (Hay 1984; McCarty 2008). Thus, local ecological usage of host plants clearly predicts the evolution of regional patterns in feeding behaviors.

We suggest that future tests of the latitudinal gradient in herbivore feeding tolerance utilize tropical herbivores that are regularly exposed to chemically-rich seaweeds (e.g., Cronin et al. 1997), such as urchins, crabs and fishes found on coral reefs (Hay 1984; Paul 1992). It is these reef herbivores that are most likely to be responding coevolutionarily to the chemical defenses of the local seaweeds. Our results also suggest that it may be fruitful to test whether the lower relative abundance of chemically-rich seaweeds within estuaries and seagrass beds relative to their frequencies on coral reefs (Hay 1984) might be directly reflected in the feeding tolerances of herbivores found in estuaries versus coral reefs.

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Variation in social foraging by fishes across a coral reef landscape

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Abstract. Coral reef landscapes are composed of a matrix of habitats that exhibit variation in complexity at multiple spatial scales. This variation has been shown to influence the composition of fish communities and influence the type and rate of species interactions. In this study, we quantified rates of social foraging bouts in single and mixed species groups across four habitat types (continuous reef, coral rubble-sand, reef edge, and spur and groove) at Conch Reef in the Florida Keys National Marine Sanctuary (USA). Our goal in dividing the reef landscape into particular habitat units was to ascertain if rates of social foraging vary in consistent ways in relation to habitat attributes. While there were no clear divisions in species composition that could be correlated with habitat type, rates of social foraging by mixed species groups were not equal. However, rates of social foraging bouts by single species groups were statistically equivalent across all habitat types. Fisher's diversity and species richness measures were not equal across habitat types, with lowest mean values in coral rubble habitat where rates of social foraging were highest. These results highlight the complexity of facilitative species interactions and suggest new lines of research to assess the demographic implications of such behaviors.

Key words: behavior, facilitation, group, mixed species, diversity

Introduction

Understanding processes that mediate local patterns in the diversity of organisms is central to developing assembly rules for community structure as well as for developing strategies for conservation. Much effort has been focused on understanding the roles that recruitment limitation (bottom up) versus predation (top down) play in structuring marine communities (see Doherty 1991; Hixon 1991). However, nested within the scope of these processes is the role positive species interactions play in mediating survivorship, growth and trophic interactions, each of which may influence local species diversity within a community.

Social foraging is a common type of species interaction in coral reef fish communities and can occur in single-species and mixed-species groups. Bouts of social foraging can focus on activities of a single focal animal (producer-scrounger foraging model) or focus on multiple individuals within a group (information-sharing foraging models; Giraldeau and Beauchamp 1999). In either case social foraging allows individuals enhanced access to prey resources, decreases search-time for patchily distributed prey, and provides increased protection from predators. Previous studies have focused on how social foraging enhances the fitness of individuals within a group (Wolf 1987, Overholtzer and Motta 2000) and how such interactions may mediate community composition and patterns of diversity (Auster and Lindholm 2002, Auster et al. 2005).

Coral reef landscapes are composed of a matrix of habitats which exhibit a gradient of spatial complexity at multiple spatial scales (Williams 1991; Sorokin 1995). This variation has been shown, in part, to influence the composition of fish communities (Williams 1991). It follows that such variation will also influence the scope of species interactions that may mediate local patterns of diversity.

In this study, we quantified the rates of social foraging bouts in relation to community composition across four distinct habitat types at Conch Reef in the Florida Keys National Marine Sanctuary (U.S.A.). Our goal in dividing the reef landscape into particular habitat classes was to ascertain if rates of social foraging vary in consistent ways in relation to habitat attributes. We hypothesized that rates of social foraging, if mediated by attributes of habitat, would be highest over comparatively low-relief topography where the trade-offs of predation risk and access to prey would be greatest.

Methods

Data were collected at Conch Reef (15-18.5 m depth) in the northeastern part of the Florida Keys National Marine Sanctuary from 11-18 September 2001, while using SCUBA. Conch Reef is part of the primarily Holocene reef tract along the continental margin off the south coast of Florida (Leichter et al. 2003), and is characterized by spur and groove formations. The reef landscape was divided *a priori* into four broad habitat classes (i.e., continuous reef, coral rubble-sand, reef edge, and spur and groove) in order to encompass the broadest range of spatial complexity. Reef edge and spur-and-groove habitat were similar at the scale of individual surveys in that patches contained a distinct transition zone from continuous reef to coral sand. However, these landscape features were assumed to be distinct due to differences in refuge from flow (enhanced shelter from flow within spur-and groove features) as well as differences in the location and direction of coral shelters for fishes. That is, fishes foraging in or above coral sand beyond reef edge habitat would only find shelter in the direction from where they came while fishes emerging over sand in spur-and-groove features could potentially find shelter across a sand patch in the adjacent spur (this distinction is based on unpublished observations by the authors of multiple species responding in these ways).

All data were collected during daylight hours (from 1 hr after sunrise to 1 hr before sunset). Survey patches were 5 m x 5 m (15-20 m depth). Patch delineation was estimated visually using natural landscape markers (e.g., particular coral colonies and formations). Surveys were 20 minutes in length. Observations were made while stationary in mid-water, away from patches, to minimize effects on behavior and movement of fishes but close enough to identify species. All species observed were identified to lowest the possible taxon and counted. However, no additional effort was made to census cryptic fishes (e.g., blennies, gobies) as this would disrupt foraging behaviors of the more active species.

Social foraging bouts (each "bout" defined as a single or mixed species group that exhibited coordinated search behavior) were enumerated as the number of species and the number of individuals per species involved in each bout. Actual feeding was not a pre-requisite for classification of a "bout" although one or more individuals within a group often did consume prey. Search behaviors involved non-linear swimming with individuals in each group focused on potential prey (e.g., sorting sediments, biting surfaces, attacks on vagile organisms). A bout ended when a group fully disbanded regardless of any exchange of individuals into or out of a group. The same fish may have been involved in multiple bouts within a census period, though the incidents of this were minimal. Number of surveys within each habitat varied slightly: continuous reef (n=15), coral rubble-sand (n=16), reef edge (n=11), and spur and groove (n=12).

Bray-Curtis similarity coefficients were calculated based on species abundances from surveys within and between habitats. Non-metric multidimensional scaling (MDS; 100 restarts) was used to evaluate the level of similarity in assemblage composition between habitat types (using PRIMER software; Clarke and Gorley 2001). Abundance data were log (x+1) transformed.

Data used to compare rates of social foraging (i.e., number of bouts per survey) were not normally distributed (Anderson-Darling tests for total, mixed-species and single species bouts all $p<0.05$). Kruskal-Wallis tests were used to determine if there were significant differences in rates of social foraging bouts between habitat types. Linear regression was used to determine if there were relationships between measures of local species diversity (i.e., species richness and Fisher's diversity of the fish community enumerated within each survey) and rates of social foraging.

Results

Overall 36.7% of species (i.e., 33 of 90 species from community census data) participated in either single or mixed species social foraging bouts, or both. Twenty seven species participated in mixed species groups (Table 1) while 22 species participated in single species bouts (Table 2). Six species participated only in single species bouts. Yellowhead wrasse *Halichoeres garnoti* and spotted goatfish *Pseudupeneus maculates* participated in the highest percentage of mixed species foraging bouts (33.8% and 21.6% respectively). Groups of bluehead *Thalassoma bifasciatum* composed the highest

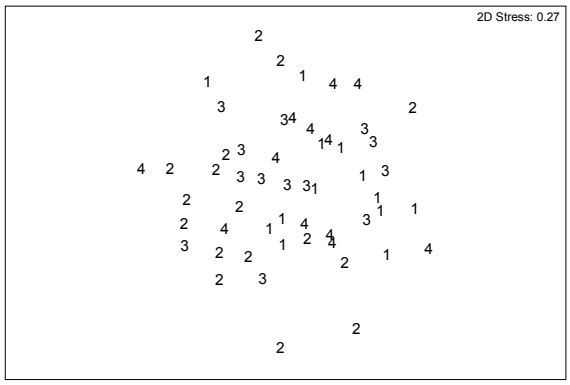

Figure 1. MDS plot illustrating similarity of species composition within and between survey sites across habitats. No habitat related associations were detected. Numbers indicate habitat type: 1 – continuous reef, 2 – coral rubble-sand, 3 – reef edge, and 4 – spur and groove.

Table 1. Percent occurrence of each species participating in mixed species foraging bouts across all surveys.

Species	Number bouts	Percent Occurrence
Chromis cyaneus	3	0.85%
Hypoplectrus nigricans	1	0.28%
Acanthurus coeruleus	6	1.70%
Thalassoma bifasciatum	17	4.83%
Hypoplectrus unicolor	7	1.99%
Clepticus parrai	2	0.57%
Acanthurus chirurgus	11	3.13%
Lutjanus jocu	1	0.28%
Chaetodon capistratus	1	0.28%
Pomacanthus paru	1	0.28%
Haemulon flavolineatum	2	0.57%
Lutjanus griseus	1	0.28%
Pomacanthus arcuatus	1	0.28%
Lachnolaimus maximus	15	4.26%
Calamus bajonado	3	0.85%
Acanthurus bahianus	5	1.42%
Scarus taeniopterus	2	0.57%
Sparisoma aurofrenatum	27	7.67%
Chaetodon sedentarius	1	0.28%
Lactophrys triqueter	9	2.56%
Pseudupeneus maculatus	76	21.59%
Sparisoma viride	2	0.57%
Scarus croicensis	20	5.68%
Aulostomus maculatus	3	0.85%
Mulliodichthys martinicus	4	1.14%
Halichoeres garnoti	119	33.81%
Ocyurus chrysurus	12	3.41%
Total	**352**	

Table 2. Percent occurrence of each species participating in single species foraging bouts across all surveys.

Species	Number bouts	Percent occurrence
Caranx rubber	4	2.07%
Holacanthus bermudensis	1	0.52%
Chromis cyaneus	15	7.77%
Acanthurus coeruleus	5	2.59%
Thalassoma bifasciatum	37	19.17%
Clepticus parrai	4	2.07%
Acanthurus chirurgus	8	4.15%
Chaetodon capistratus	16	8.29%
Lutjanus griseus	1	0.52%
Pomacanthus arcuatus	2	1.04%
Serranus tigrinus	1	0.52%
Lachnolaimus maximus	3	1.55%
Acanthurus bahianus	15	7.77%
Sparisoma aurofrenatum	5	2.59%
Chaetodon sedentarius	13	6.74%
Holacanthus tricolor	1	0.52%
Canthigaster rostrata	2	1.04%
Chaetodon ocellatus	12	6.22%
Pseudupeneus maculatus	15	7.77%
Scarus croicensis	12	6.22%
Halichoeres garnoti	18	9.33%
Ocyurus chrysurus	2	1.04%
Total	**192**	

percentage of single species bouts (19.7%) but all other species represented less than 10% each of the remaining bouts.

Results of MDS analysis (Fig. 1) indicate that there were no clear divisions in species composition that could be attributed to habitat type, although not all species occurred in all habitat types.

Comparisons of rates of social foraging based on total and mixed species bouts showed that rates of social foraging across habitats were not equal (Kruskal-Wallis tests, df = 3, H = 14.78 and 16.94; p = 0.002 and 0.001 respectively). For both comparisons, continuous reef had the lowest mean rank of all habitats and coral rubble-sand had the highest (Fig. 2 top and middle). Paired comparisons showed that coral rubble-sand had a significantly higher rate of social foraging bouts ($p<0.05$) than the other three habitat types which were not statistically different from one another. There were no significant differences in the number of single species bouts and habitat type (Kruskal-Wallis test, df = 3, H = 5.33, p = 0.149 adjusted for ties; Fig. 2 bottom).

There was no linear relationship between the total number of social foraging bouts and species richness across all surveys (ANOVA, $df_{1,52}$, F = 0.01 p = 0.91). However, when species abundances as well as richness were taken into account there was a significant linear relationship between Fisher's diversity and total bouts (ANOVA, $df_{1,52}$, F = 5.25 p = 0.03). Neither Fisher's diversity nor species richness measures were equal across habitat types (Kruskal-Wallis tests, df = 3, H = 14.15 and 10.58; p = 0.003 and 0.015 respectively) with lowest mean values in coral rubble habitat where rates of social foraging were highest (Fig. 3).

Discussion

The benefits to individuals within a foraging group generally are considered to include enhanced vigilance to predation threats and enhanced access to prey (Lukoschek and McCormick 2002). In this study we found variable rates of social foraging among habitats within a coral reef landscape despite

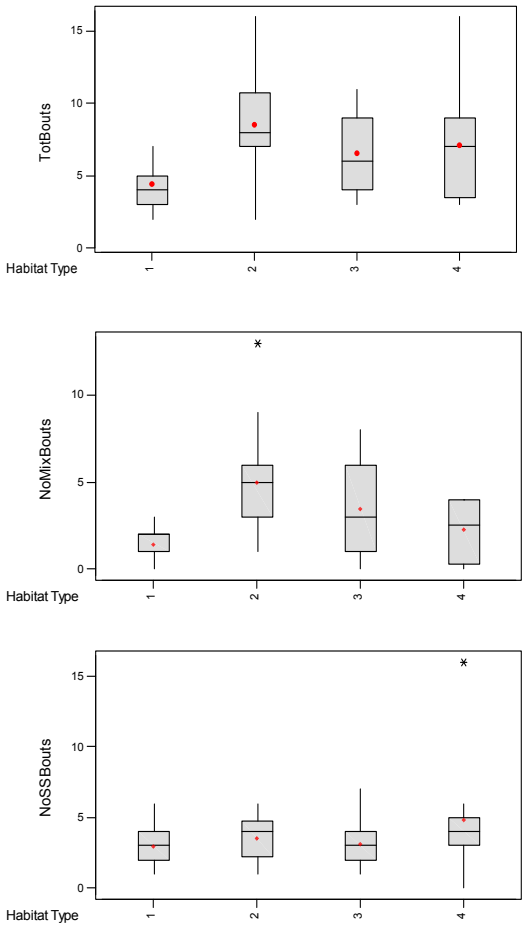

Figure 2. Boxplots of total (top), mixed species (middle) and single species (bottom) bouts per survey. Habitat types as in Figure 1. (The center-line through each box marks the median value and the solid dots mark the mean. Top and bottom of each box mark the bottom of the third and the top of first quartile of the data, respectively. The top whisker extends to the highest value in the top quartile and the bottom whisker to the lowest value in the first quartile. Stars indicate statistical outliers.)

similarity in community composition across habitats. These results suggest that structural attributes of habitat such as spatial complexity have a role in mediating behavior that balances the time and space constraints related to prey acquisition and predator avoidance.

Our *a priori* hypothesis of higher rates of social foraging in comparatively low complexity habitats was confirmed. Rates of social foraging were highest in coral rubble-sand habitats, where cover from predation was low and prey organisms are assumed to be patchy in distribution. The increased number of social foraging bouts was indicative of enhanced vigilance against threats of predation. Multiple bouts often resulted from a group breaking off and then reforming following a strike by a predator. Though predator strikes were frequent, no successful strikes were observed against individuals participating in social foraging bouts over the course of the study. Conversely, the number of social foraging bouts was lowest over continuous reef habitat where cover from predation and potential prey was ubiquitous. Here the benefits to an individual's participation in a social foraging event are assumed to be comparatively low.

It is interesting to note that fishes in this study area exhibited a lower percentage of social foraging activities than studies elsewhere. For example, 52% of species (i.e., 34 of 65 species) from community surveys on reefs off Bonaire, Netherlands Antilles, participated in mixed or single species foraging groups (Auster and Lindholm 2002). Barber and Auster (in prep) demonstrated that approximately 45% of non-cryptic reef fish species at sites in the Gulf of California and the Great Barrier Reef occurred in mixed and single species foraging groups. While all surveys were conducted within approximately the same depth range, patterns related to latitudinal gradients and relationships to the regional species pool remain to be investigated.

Observations of foraging groups elsewhere in the Caribbean, Gulf of California, Coral Sea, and Indian Ocean revealed they can be composed of

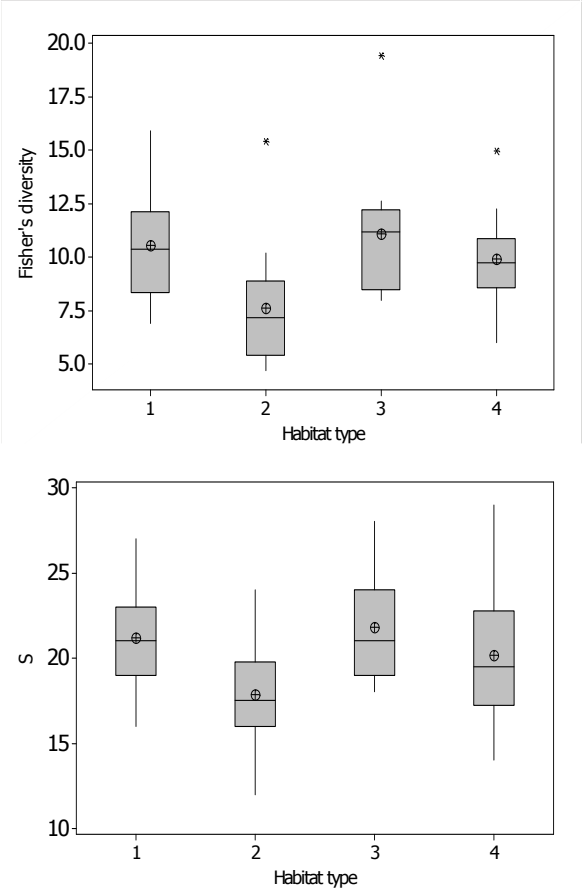

Figure 3. Boxplots of Fisher's diversity (top) and species richness (bottom) by habitat type. See legend of Figure 2 for explanation.

species representing a wide range of trophic guilds (i.e., planktivores and herbivores through piscivores; sensu Bohnsack et al. 2002) while others were limited to a particular trophic groups (Auster and Lindholm 2002, Auster 2005, 2007, Barber and Auster in prep, Auster unpublished data). Barber and Auster (in prep) showed that while all trophic guilds were represented in foraging groups at Caribbean (i.e., Bonaire) and Coral Sea (northern Great Barrier Reef) study sites, the proportions of guilds represented in foraging groups in contrast to the local community varied. While the trophic guild composition of social foraging groups at the Caribbean site reflected proportions in the local community, the guild composition of foraging groups differed significantly from the community at Coral Sea sites. Higher than expected proportions of planktivores, browsers and macro-invertivores were observed in mixed-species groups while piscivores and micro-invertivores were under-represented. However, a mechanism to explain such patterns in terms of costs and benefits remains to be discerned and will be critical for understanding the link between facilitative behaviors and population responses of component species (Auster et al. 2005, Barber and Auster in prep).

Species interactions can be explicitly linked to local patterns of diversity. For instance, a study of mixed flocks of neotropical birds suggested that such behavior may contribute to enhanced richness of rainforest avifauna (Powell, 1989). The author showed that smaller bird species in mixed species flocks foraged within a larger spatial range than when foraging singly, and suggested that each species underutilized available prey resources, thus allowing coexistence of species with high niche overlap. In the present study, local fish diversity was positively correlated with rates of group foraging, as it was at reefs off Bonaire (Auster and Lindholm 2002). In the Bahamas the combination of predator-prey and competitive interactions between reef fishes was shown to mediate patterns of local abundance of reef fishes (Carr et al. 2002). However, the role of positive (facilitative) species interactions in developing these patterns was not addressed.

Ultimately, investigating such interactions in fishes at a diversity of locations can lead to a better understanding of how behavioral interactions mediate trophic interactions and community composition. In the short term we need to better understand how quantification of such interactions can enhance our ability to evaluate the status and ecological integrity of reef fish communities.

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Factors influencing coral recruitment patterns in the Sulu Sea marine corridors

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Abstract. Coral recruitment studies provide crucial information on a reef's capacity to recover from disturbances such as storms, coral bleaching, or destructive fishing. The Sulu Sea is at the apex of a "coral triangle", yet little is known on coral recruitment or reef resilience in this area. This study aimed to determine patterns in the abundance and composition of early coral recruits in three major marine corridors of the Sulu Sea. Five hundred nineteen terracotta tiles were deployed for six months along 50-m transects in 33 sites along the Cagayan Ridge, Balabac Islands, and Verde Passage. Results show significantly higher mean recruit density in the Balabac Islands, where destructive fishing methods continue to be used, than in the Cagayan Ridge, which is a protected area. At a smaller scale, density patterns of early recruits show no direct correlations with the adjacent benthic community. High early recruit density was found in sites with derived high entrainment by currents, suggesting that physical rather than biological factors play a major role in determining early recruitment at the scale of both corridors and sites.

Key words: Coral recruitment; Sulu Sea

Introduction

The Indo-Malay-Philippines "coral triangle" possesses the highest marine biodiversity in the world (Hoeksema 2007), and the Sulu Sea in central Philippines has among the highest concentrations of species per unit area in this region (Carpenter and Springer 2005). Reefs in the Sulu Sea are important not only for their high biodiversity and unique geology, but also as potential sources and sinks of larvae for other reefs, thus maintaining marine biodiversity in other Philippine inland seas (Alcala 1993). Understanding reef community dynamics, particularly recruitment processes, is crucial in determining reef recovery rates from perturbations due to human and natural disturbances (Hughes et al. 2005; Coles and Brown 2007).

Larval supply is determined by various factors, such as spawning season of adult coral species in the community and proportion of fecund adults (Hughes et al. 2000; Adjeroud et al. 2007). The amount and composition of larvae produced, however, can be very different from a reef's actual juvenile community structure. Studying larval settlement patterns may instead provide more insight into early recruitment dynamics. Coral recruitment patterns depend on a reef's physical and biological attributes, such as size (Glassom et al. 2006), morphology and existing community (Mumby et al. 2007; Norström et al. 2007; Perkol-Finkel and Benayahu 2007), as well as speed and direction of ocean currents. These influence how and where larvae are distributed, and eventually settle (Banks and Harriott 1996; Chen 1999; Taylor and Pearce 1999). The abundance and composition of recruits also change with increasing distance from the reef, a function of both current velocities and differing reproductive strategies among coral species (Sammarco and Andrews 1989).

This study sought to identify patterns in coral recruit abundance and composition in three Sulu Sea marine corridors at two spatial scales, i.e. within and among corridors. Possible physical and biological factors that might explain these patterns were investigated to gain insights on what processes influence the relative resilience and recovery of reefs from disturbances.

Material and Methods

Study site and data collection

The study was conducted in the major marine corridors of the Sulu Sea: Verde Passage in the north, Cagayancillo Islands and Tubbataha Reefs, comprising the Cagayan Ridge, in the central area, and Balabac Islands in the southwest (Fig. 1).

Approximately 16 terracotta tiles, each measuring 10 cm x 10 cm x 1.2 cm, were deployed along a 50-m transect in each of 33 sites. Sets of four tiles were nailed individually to the substrate at roughly 12-m intervals along each transect (Mundy 2000; Quibilan and Aliño 2004). The tiles were deployed at the onset of the northeast (NE) monsoon season in October 2006, and retrieved in April and May 2007, before the onset of the southwest (SW) monsoon season. Data gathered, therefore, represents recruitment for the NE

monsoon season. This season also covered the peak coral spawning months in the Philippines (Bermas et al. 1992; Atrigenio and Aliño 1994).

Upon retrieval, the tiles were air-dried, then examined under a stereoscope. The number of coral recruits on the top, undersurface, and sides of the tiles were counted and identified to family level according to the guide provided by Babcock et al (2003). Due to natural disturbances, not all deployed tiles were recovered.

Data analyses

Differences in mean recruit abundance at both site and corridor level were compared using the Analysis of Variance (ANOVA). Abundance data were fourth root-transformed prior to statistical analysis to conform to the Cochran C, Hartley, and Bartlett tests for homogeneity of variances. Tukey's Unequal N Honestly Significant Difference was used as post-hoc test.

To determine differences in recruit composition among sites and corridors, abundance data for each coral family were simultaneously compared among sites using the Analysis of Similarities (ANOSIM) based on the Bray-Curtis Similarity index (Clarke and Warwick 2001). Recruit density and composition data in each site were tested for correlations with geographical location and benthic composition of the surrounding reef using Pearson product-moment correlation, and Similarity of Percentages (SIMPER) based on Spearman rank correlations. Data collected by Deocadez et al. (this symposium's proceedings) on live benthic cover of the adult reef community using video transects was used for this analysis.

Results

Corridor Level

Mean recruit density was significantly higher in Balabac Islands than in the central corridors of Tubbataha Reefs and the Cagayancillo Islands (Fig. 2). Verde Passage did not differ significantly from either Balabac or Cagayancillo. Balabac also had the largest range in number of recruits per tile, while Tubbataha had the smallest (Fig. 2).

Pocilloporids dominated in Balabac and Verde, while acroporids dominated in the central Sulu Sea (Fig. 1). Poritids and other families each comprised less than 10% of recruits in all corridors. Sixty-nine percent of the recruits were found on the sides of the tiles, 19% on the undersurface, and 12% on the top. This trend was consistent in each corridor.

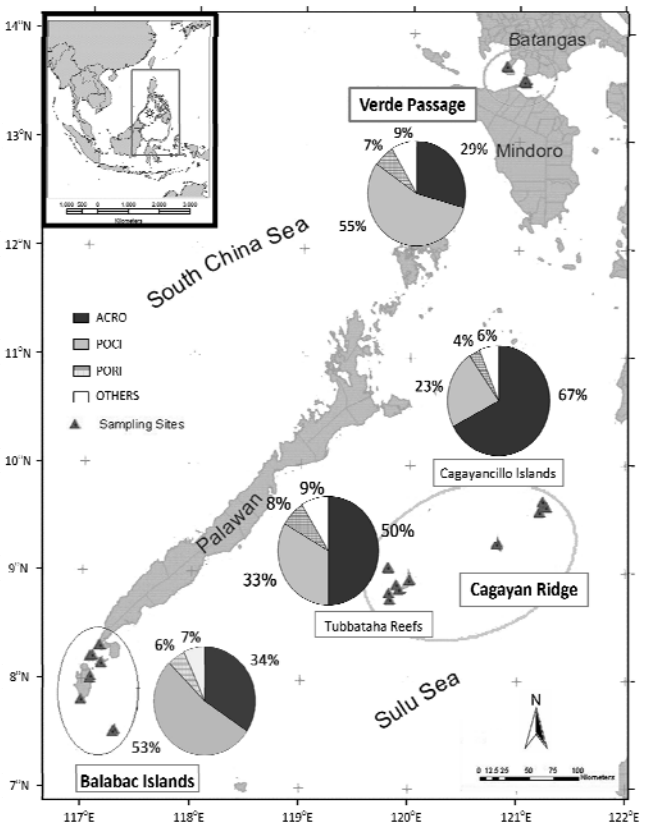

Figure 1: Location map of sampling sites with mean recruit density and composition per corridor. Each corridor spanned an area ranging from ≈300-3000 km^2, with Verde Passage being the smallest and Balabac Islands being the largest, due to its offshore islands. Adjacent corridors ranged from being ≈150-500 km apart. Each site covered an area of ≈100 m^2. Adjacent sites within corridors ranged from being ≈50 m-45 km apart. There were 6 sites in Verde, 8 in Tubbataha, 9 in Cagayancillo, and 10 in Balabac.

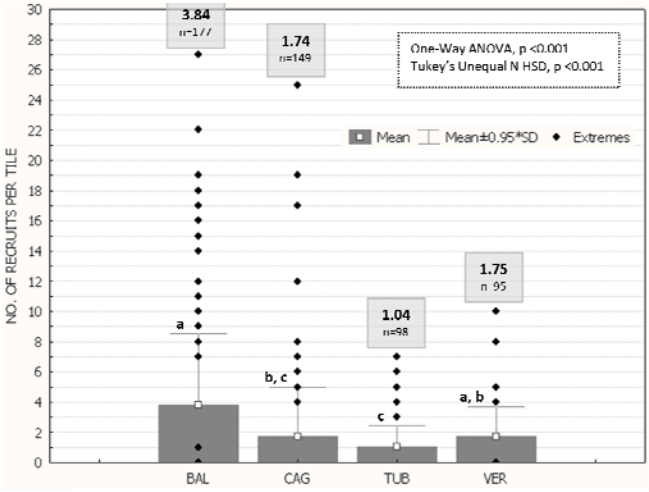

Figure 2: Mean recruit density with range of extreme values per corridor. Bars with the same letters are not statistically different.

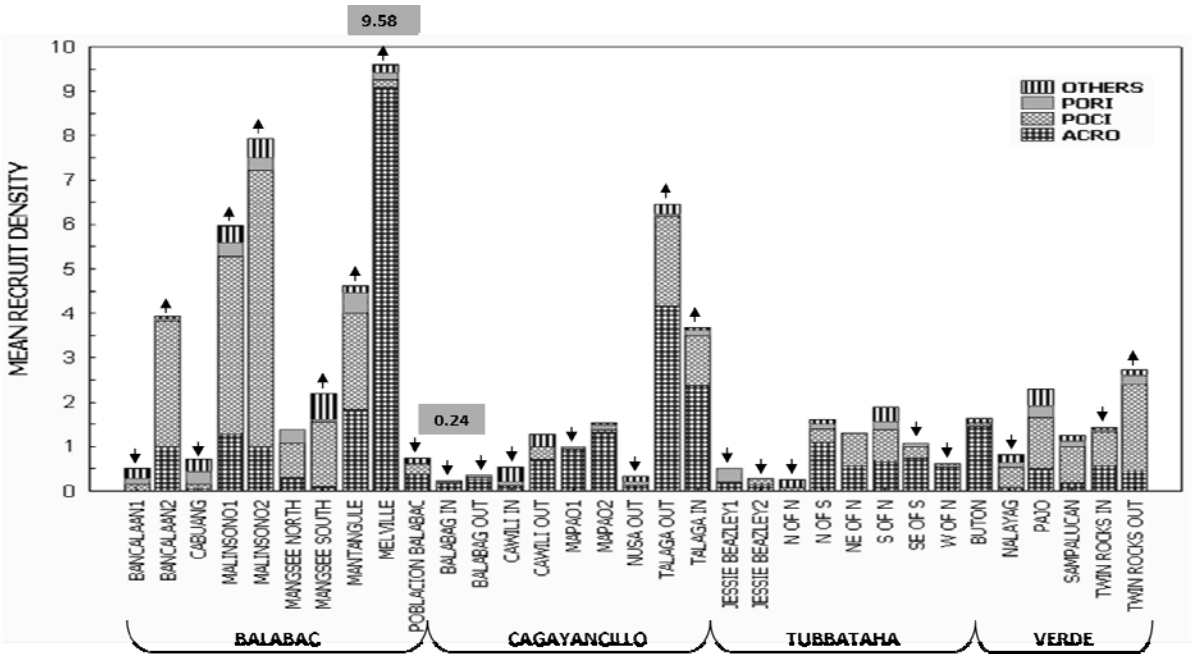

Figure 3: Mean recruit density and composition per site. Bars with up arrows indicate sites not statistically different from Melville. Bars with down arrows indicate sites not statistically different from Balabag In. Sites with no arrows are not statistically different from both Melville and Balabag In.

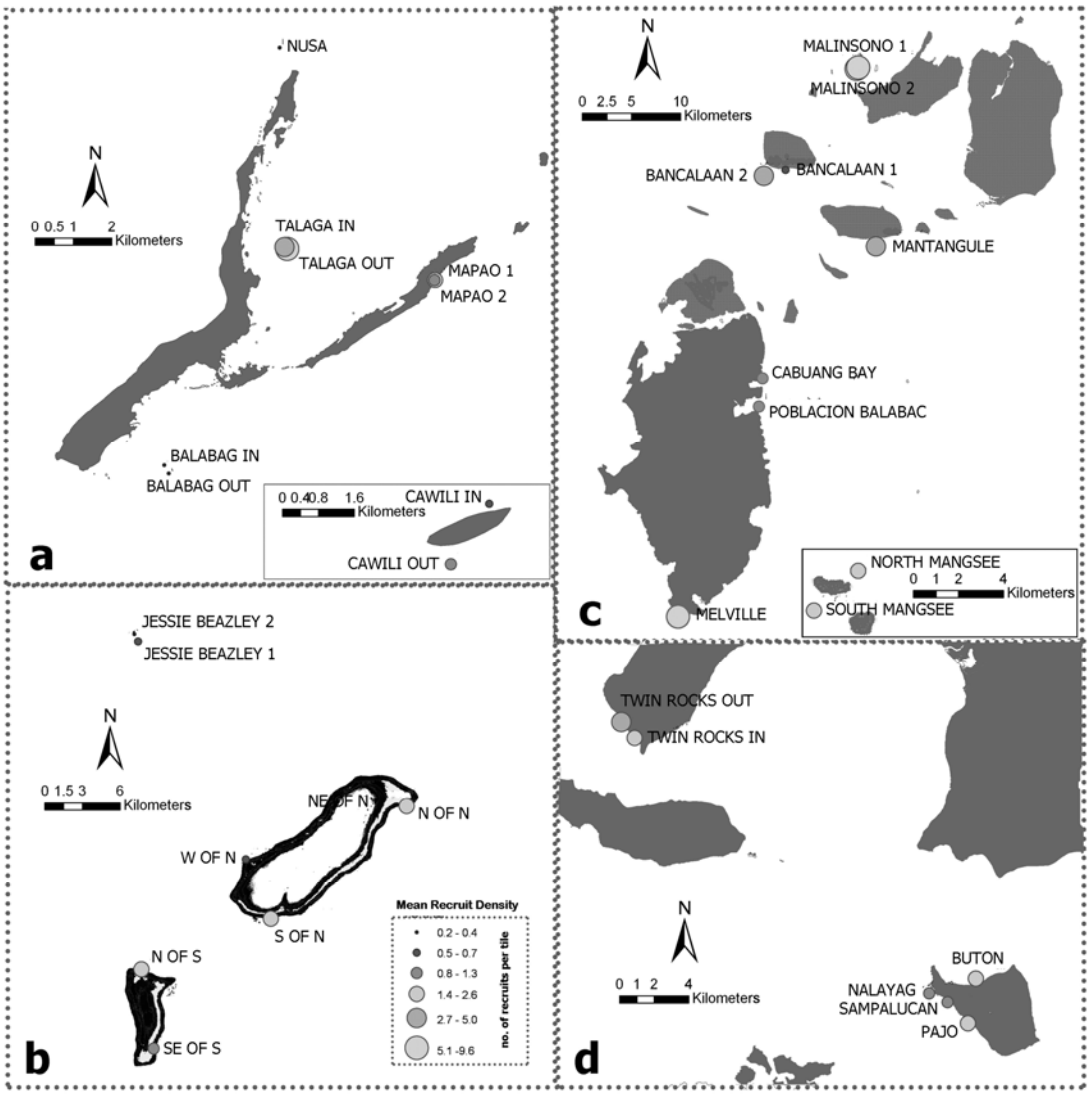

Figure 4: Map of mean recruit density per site, within corridors. a) Cagayancillo Islands [inset: Cawili Island, approx. 50 km SW] b) Tubbataha Reefs c) Balabac Islands [inset: Mangsee Islands, approx. 45 km SE] d) Verde Passage.

Site Level

Melville in Balabac had the highest mean recruit density among all sites (Fig. 3). Balabag In, found in Cagayancillo, had the lowest. Sites with high recruit density (i.e., not statistically different from Melville but statistically different from Balabag In) tended to be in areas between islands or in island wakes, with the possible presence of eddies (pers. observations from derived current circulation; see Vilanoy et al., this symposium's proceedings).

In Balabac, sites less than 500 m from the nearest shoreline (Cabuang, Poblacion, Bancalaan 1) had significantly lower mean recruit density than sites farther offshore. In the central Sulu Sea, outer reefs generally had lower recruit density than those closer to islands or other geological barriers. (Fig. 4)

Melville and Malinsono 1 in Balabac, and Talaga In and Out in Cagayancillo had significantly higher acroporid recruitment than all the other sites. Pocilloporid recruits generally dominated in sites on the western side of the islands, as opposed to the eastern side, in both Balabac and Verde (ANOSIM R=0.417, p=0.006). No apparent patterns in recruit composition were found in the central Sulu Sea sites in relation to their location on the reef.

Percent cover of adult *Acropora* along the transect, and the number of acroporid recruits on the tiles were not found to be correlated (Pearson product-moment correlation, r=-0.1172). Neither were recruit density and composition found to be associated with any other benthic category or with over-all benthic composition in each site (Spearman rank correlation, ρ_s=0.086).

Discussion

Differences in patterns of recruit abundance among the marine corridors suggest that individual physical factors have different degrees of influence on settlement rates. Distinct features of each corridor may magnify or reduce these effects.

Higher mean recruit density occurs in the Balabac Islands despite continuing destructive fishing activities. Contrary to this, Tubbataha Reefs and Cagayancillo Islands have relatively lower early recruit abundance despite being protected for years. Two factors may be most crucial: larval supply and entrainment potential.

Balabac is at the junction of two possible sources of coral larvae, the South China Sea and the Sulu Sea, which potentially increases its larval supply. Monsoonal wind-driven currents interacting with the relatively more complex configuration of islands in Balabac may result in the aggregation or entrainment of coral larvae, increasing their chances of settlement. As previously noted, sites with high recruit density, regardless of which corridor they were found in, tended to be between islands or in island wakes, which have high larval entrainment potential (Magno and Villanoy 2006). The greater number of islands in Balabac may also create more heterogeneous microhabitats with varying suitability for larval settlement, as suggested by the large range in recruit densities within this corridor compared to other corridors. This may explain why the Tubbataha Reefs and Cagayancillo corridors have the lowest mean recruitment, being reef slope sites located at least 200 km from other reef agglomerations, such as those in the Visayan seas and Palawan mainland.

Recruitment patterns differ between coastal waters and open seas because of very different hydrodynamics created by factors such as bathymetry and stratification nearer to shore (Pineda et al. 2007). Sammarco and Andrews (1988,1989) showed that highest recruitment occurs in the area of a reef with the lowest flushing rate. On the other hand, slower currents in inner reefs or the lee of islands tend to have higher sedimentation rates, which may inhibit recruitment (Murray et al. 1977; Connell et al. 1997). Ideal conditions for larval settlement may therefore be a complex topography that produces eddies which increase the larvae's residence time, in combination with strong currents that constantly flush out sediment. These findings highlight how major consideration must be given to current patterns in prioritizing sites for protection.

Apparent patterns in recruit composition at the corridor level (N-S) and site level (E-W) suggest that monsoonal wind-driven currents may determine the sources and sinks of larvae, depending on the site's geographical location in relation to the prevailing wind. Such monsoonal variation in recruit composition was observed in Palawan shelf reefs in the central Sulu Sea, where acroporids dominated during the SW monsoon, and pocilloporids during the NE monsoon (Quibilan and Aliño 2004). These patterns may also be due to seasonal currents interacting with species-specific peaks in coral spawning, as well as latitudinal differences (Hughes et al. 2002).

The lack of concordance between recruit and adult hard coral composition is not unusual, as seen in other studies (e.g. Banks and Harriott 1996; Hughes et al. 1999). This affirms the importance of post-settlement events in determining adult community structure following both anthropogenic and natural disturbances, such as crown-of-thorns outbreaks, storms and thermal anomalies resulting in bleaching (Arceo et al. 2001; Quibilan and Aliño 2004; Victor 2008).

Acknowledgements

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Differences in coral-reef fish assemblages between mangrove-rich and mangrove-poor islands of Honduras

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Abstract. Visual surveys of coral-reef fish were conducted on two islands (and associated cays) off the Caribbean coast of Honduras, Cayos Cochinos (mangrove-poor) and Utila (mangrove-rich). Local populations on both islands exploit all marine habitats, and Utila suffers from higher fishing pressure compared to Cayos Cochinos, the latter having a conservation plan and no-take zones. Nine sites were visually surveyed in the mangroves to identify nursery species. Coral reef surveys consisted of eight randomly selected 50m transects at each site (six sites per island) where fish abundances, microhabitat percent cover, and rugosity were recorded. Findings from this study reveal that adult coral-reef nursery fish assemblages differ between the two islands. Out of 13 coral-reef fish whose juveniles are found in mangroves but absent from reefs, eight species had significantly higher adult coral-reef fish abundances on Utila than Cayos Cochinos. Canonical Correspondence analysis found that rugosity and percent algae explained a small but significant variation in fish assemblages. This correlation most likely explains the occurrence of increased adult non-nursery species abundance on Utila. However, a higher percentage of adult nursery species (compared to non-nursery species) had higher abundances on the surrounding reefs of Utila indicating the importance of the presence of mangroves.

Key words: Coral-reef fish, Mangroves, Nursery, Microhabitat.

Introduction

Shallow marine biotopes are thought to act as nurseries for many species of tropical fish (Dahlgren et al. 2006). Nurseries are believed to disproportionately enhance numbers of juveniles that move into adult habitats (Beck et al. 2001). Nursery reef fish species are species that are obligate to habitats other than coral reefs while in the juvenile stage. Studies have shown that mangrove and seagrass habitats provide vulnerable newly settled fish with appropriately sized food and refuge from predators (Laegdsgaard and Johnson 2001; Verweij et al. 2006).

Studies have focused on juvenile fish assemblages in mangroves and seagrass beds (Nagelkerken et al. 2000; Dorenbosch et al. 2006; Hindell and Jenkins 2004; Lugendo et al. 2006), and very few have actually compared adult populations in areas with and without mangrove nurseries (Nagelkerken et al. 2002; Mumby et al. 2004).

Past mangrove studies in the Caribbean have made pair-wise comparisons between two islands (mangrove-rich vs. mangrove-poor), to indicate the importance of mangroves as nursery sites. Island comparisons have shown higher abundances of nursery species on islands inhabited by mangroves (Nagelkerken et al. 2002; Mumby et al. 2004).

The aim of this study is to find which coral-reef fish juveniles reside in Honduran mangroves, and to compare these nursery species with control species (non-nursery) abundances between mangrove-rich and mangrove-poor islands. Previous studies have identified covarying factors that influence reef fish populations such as size of coral reefs and dominant coral species; however, this is the first study to include microhabitat variables of reef structure in influencing the adult fish structure. By including these variables, it will be possible to better isolate the importance of mangrove nursery grounds in determining adult reef fish populations**.**

Material and Methods

Study Area

Sampling and surveying were conducted on the surrounding reefs and mangroves of Utila, Bay Islands and the Cayos Cochinos Islands (Cayos C.), Honduras (Fig. 1). Utila, which has 13 cays, is the southernmost island in the Bay Island archipelago and is located 29 km off the coast of Honduras. Utila is dominated by mangroves (nearly 66% coverage). The mangroves, *Rhizophora mangle* and *Avicennia germinans,* dominate two large lagoons on the south side and mangrove stands on the north side of the island.

Cayos C. consists of Cayos Menor, Cayos Mejor and 13 smaller coral cays. The islands are located southeast of the Bay Islands, 18.5 km from the mainland. Tropical forests cover both Cayos Menor and Mejor, while most of the cays are sandbars. In contrast to Utila, Cayos C. has a distinct absence of mangrove lagoons and supports only two very small mangrove stands with lengths of 100 and 150 meters.

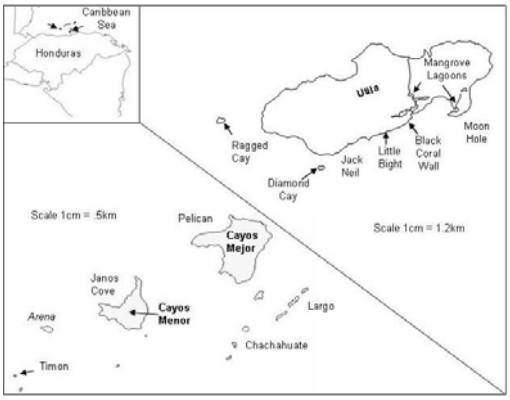

Figure 1: Map of Utila and Cayos Cochinos, Honduras, showing the 12 sites surveyed.

Fish surveys on coral reefs

This study used underwater visual census to make relative comparisons of fish abundances on both islands. Six sites per islands (Utila and Cayos C.) were surveyed. Sites were chosen based on three conditions (1) varying distance to mangroves, (2) reef flat shallower than nine meters (to eliminate any effects depth may have on fish assemblages), and (3) reef large enough to encompass replicate transects.

Within each site, eight non-overlapping 50 meter transects were laid out randomly (separated by >10m). Perpendicular distance and direction between transects were predetermined using random numbers. Each transect was laid out parallel to the reef wall, ensuring that the tape remained within a depth range between four and nine meters (local reefs form walls at depths greater than nine meters). Divers waited five minutes after deploying the tape for the fish to resume normal activity before beginning the survey (Tolimieri 1995). All fish (classified into life history stages), with the exception of small cryptic fish (e.g. gobies and blennies), were visually surveyed within one meter on each side of the tape (total width of 2m) and 2.5 meters above the tape. Life history stages (juvenile vs. adult) were defined by de la Moriniere et al. (2003) and FishBase (2008) (1/3 max adult size), while the initial phase stage was defined by distinct coloration differences (Humann and Deloach 2002).

Lutjanid, Chaetodonid, Haemulid, Scarid, and Serranid adults (large fish or fish with large home ranges) were resurveyed using a larger transect of five meters width. Previous to the study, divers spent two weeks practicing fish identification and experimenting with different transect areas to improve survey accuracy. Multiple divers were used only after survey records matched principle diver's records. Sites surveyed were rotated, to randomize diurnal and lunar variables. All surveys were performed between 0900 and 1600 to ensure consistency in fish activity and presence (Colton and Alevizon 1981).

Fish surveys in mangroves

To establish which coral-reef species use mangroves as a juvenile nursery (aka nursery species), nine mangrove sites (one on Cayos C., two on the northside of Utila, and six in Utila's western mangrove lagoon) were visually surveyed using snorkeling gear. In each site, 30x1m transects, with 10 meters separation, were measured out and marked with flagging tape before surveying.

Habitat and Rugosity

In order to provide insight into other potential mechanisms underlying anticipated differences in the fish communities between islands, microhabitat characteristics within every transect were measured using the point-contact technique (Caselle and Warner 1996). At every one meter interval on the tape, substrate type was recorded and live habitat was identified to species level (soft coral and algae were only identified to genus level due to lack of taxonomy expertise). Non-living habitat was recorded as sand, dead coral, or coral rubble (broken coral, mostly *Acropora* spp.)

Rugosity, a measure of the variation in height of physical surfaces along a linear extent of habitat, is often employed as one of the most important measurements of habitat complexity. On each transect, rugosity was measured using the traditional chain-transect method, laying a chain over the contours of the reef and measuring the resulting length (Risk 1972). The chain-transect rugosity is the ratio of contoured distance to the linear distance.

Analysis

Coral-reef fish assemblage of Utila and Cayos C. was analyzed using a Bray Curtis multivariate scatterplot and ANOSIM analysis from Community Analysis Package (Copyright Pisces Conservation LTD) statistical software. Data were first square-root transformed.

Fish abundances were statistically compared between Cayos C. and Utila. Comparisons were made using SPSS statistical software; a Mann-Whitney U-test was used on square-root transformed data.

One-way ANOVA, without data transformation was used to compare rugosity and microhabitat

percentages between the two island sets. In the case of significance, Bonferroni post-hoc tests were used to compare between sites. Canonical Correspondence analysis (CCA) and Monte Carlo tests (Environmental Community Analysis, Copyright Pisces Conservation LTD, statistical software) were used to compare microhabitat percent cover and rugosity to fish assemblages. To compare rugosity and microhabitat with abundances of individual fish species, Spearman-ranked correlations (SPSS) were used.

Results

Comparison of adult coral-reef fish assemblages between islands

Utila and Cayos C.'s multivariate plots of assemblages of fish nursery species on coral reefs show a significant difference (Fig. 2, ANOSIM, $p < 0.001$, $R = 0.104$) between the two islands.

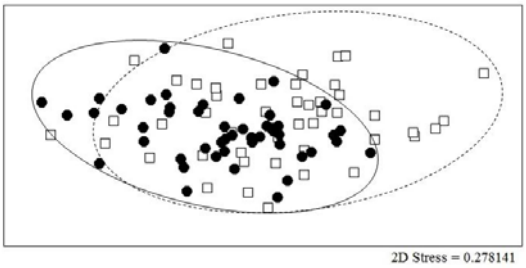

Figure 2: Multidimensional scaling ordination plot (Bray-Curtis dissimilarity coefficient) of fish assemblages (adult nursery species on coral-reefs) on Utila and Cayos C.
(□: Cayos Cochinos, ●: Utila).

Comparisons of average adult abundances on coral reefs (data from 5m transect width)

Of the 13 nursery species whose juveniles were found primarily in mangroves, eight fish species had significantly higher abundances on mangrove-rich Utila's coral reefs (Fig. 3). These species include butterflyfish *Chaetodon striatus* ($p < 0.01$), *C. capistratus* ($p < 0.01$), and *C. ocellatus* ($p < 0.001$), the parrotfish *Sparisoma chrysopterum* Initial Phase ($p < 0.05$), the snapper *Lutjanus apodus* ($p < 0.001$) and grunts *Haemulon carbonarium* ($p < 0.001$), *H. flavolineatum* ($p < 0.001$), and *H. sciurus* ($p < 0.001$).

These trends were present when broken down to site-to site comparisons. For seven species with significantly higher abundances, at least two sites on Utila (actual sites differed per species) had significantly higher abundances than three sites on Cayos C. ($p < 0.05$). *C. striatus* was the only species whose abundance showed no significant difference between Utila and Cayos C.'s individual sites.

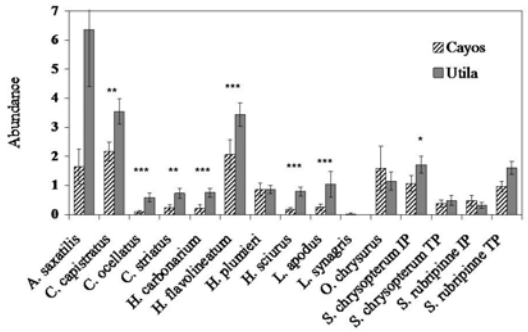

Figure 3: Average abundance per transect (±SE) of adult coral-reef fish whose juveniles were found in the mangroves. (*: $p<.05$; **: $p<.01$; ***: $p<.001$) (IP: Initial Phase; TP: Terminal Phase).

Comparing non-nursery and nursery species between islands

Some non-nursery adult fish species had higher abundances on Utila as well. However, a larger percentage of nursery species than non-nursery species had higher abundances on Utila (Fig. 4)

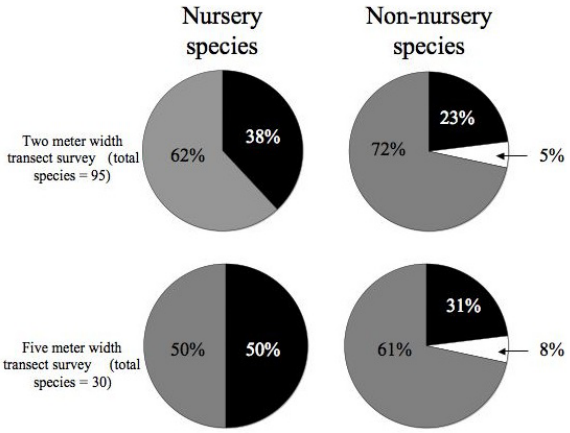

Figure 4: Pie charts depicting nursery and non-nursery fish abundance percentages for both the two meter and five meter width transect surveys (■ Percent species whose abundances were significantly higher on Utila , □ abundances lower on Utila, ■ No significant difference in abundances between Utila and Cayos C.)

Coral Reef Microhabitat and Rugosity

The point-contact microhabitat survey resulted in nine genera of algae, 13 genera of sponges, 17 genera of live hard coral, and eight genera of soft coral. Algae covered the largest percentage (Cayos C.: 40%; Utila: 33%) of transects on both islands; *Lobophora, Dictyota,* and *Halimeda* spp. dominated. Cayos C. had a significantly higher percentage of algae than Utila ($p < 0.05$), while Utila had a significantly higher percent cover of non-living coral than Cayos C. ($p < 0.001$). Percent cover of hard coral and soft coral, approximately 17% and 12% respectively, was not significantly different between the islands.

Average rugosity ratios for Utila and Cayos C. were 1.37 (SE ± 0.4) and 1.41 (SE ± 0.03),

respectively. Ratios between islands were not significantly different ($p = 0.370$). In addition, variation in depth (4-9m) produced no significant effects on fish assemblages.

Canonical Correspondence Analysis showed that algae (4.98% of variance) and rugosity (4.45% of variance) were the two best explanatory factors explaining variability in fish assemblages (see Fig. 5 for the ordination plot). Monte Carlo randomization tests demonstrated that the plotted axes were significant at the 0.05 level.

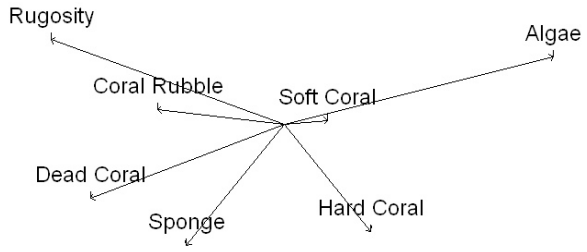

Figure 5: Canonical Correspondence Analysis Ordination plot of environmental vectors in correspondence to adult nursery fish assemblages. Length of vectors represents strength of explanatory variable, while direction of vector shows positive/negative correlation with other vectors.

Spearman-Ranked Correlations on substrate composition and rugosity did not reveal any clear correlations between these explanatory measurements and "mangrove-dependent" fish abundances with the exception of *Abudefduf saxatilis* (Utila and Cayos *C.* $p < 0$.051).

Discussion

Comparison of adult coral-reef fish assemblages between islands

Studies in Belize and Mexico found that the structure of Caribbean reef fish communities on reefs was significantly different between mangrove-rich and mangrove-poor sites (Mumby et al. 2004). These authors concluded that mangroves were the "dominant factor structuring reef fish communities."

Furthermore, the presence of mangroves in the Belize/Mexico studies were significantly correlated with the biomass of six species. *Scarus iserti* (striped parrotfish), *Haemulon sciurus* (blue-stripped grunt), *Haemulon flavolineatum* (French grunt), *Haemulon plumieri* (white grunt), *Ocyurus chrysurus* (yellow-tail snapper) and *Lutjanus apodus* (schoolmaster grunt) had biomass increases > 42% (Mumby et al. 2004). Two other studies have surveyed fish on coral reefs in the presence/absence of mangroves. In a study performed by Dorenbosch et al. (2003), sites adjacent to seagrass/mangrove bays had significantly higher densities of *H. sciurus, O. chrysurus* and *L. apodus.* Nagelkerken et al. (2002) compared fish assemblages on islands with and without nursery lagoon; *H. sciurus*, *O. chrysurus, L. apodus,* and *S. iserti* were highly dependent on mangrove nurseries. Likewise, the current study found significantly higher abundances of *H. sciurus, H. flavolineatum,* and *L. apodus* on Honduran reefs adjacent to islands with mangroves (Utila). *L. apodus* was the most common juvenile species found in the majority of mangrove studies performed in the Caribbean; therefore, mangroves have been deemed a vital habitat for this species (Nagelkerken et al. 2000; de la Moriniere 2003; Chittaro et al. 2005; and Verweij et al. 2006).

Since approximately 50% of the juveniles of *S. iserti* were found on coral reefs (53% on Cayos C., 40% on Utila) (data from surveys of 2m width transects), it was not considered to be a nursery species. Unlike previous studies, *S. iserti* adults were not found to have higher densities on mangrove-rich sites. In fact, their abundances were significantly lower at coral reef sites near mangroves.

Although Nagelkerken et al. (2002) concluded that *Chaetodon capistratus* and *Sparisoma chrysopterum* did not depend on mangroves, the present study found significantly higher abundances on Utila's coral reefs. Therefore, the dependence of these two species on mangrove nurseries is not a fixed factor. The current study also found significantly higher abundances of both *Chaetodon striatus* and *C. ocellatus* adults on the Utila. Nagelkerken et al. (2002) did not survey these two species. Nursery species *Abudefduf saxatilis, Haemulon plumieri,* and *Ocyurus chrysurus* were not significantly different between islands.

Coral-reef fish abundance explanatory variables

Although this study focuses on the positive effects mangroves have on nursery species abundances, many different explanatory variables (fishing pressures, rugosity, microhabitat, recruitment differences) may affect fish assemblages. Although local artisanal fishermen fish on both Cayos C. and Utila, Cayos C. has reduced fishing pressure due to a marine reserve conservation plan therein. On Cayos C., commercial fishing is illegal, fishing gear is regulated, and no-take zones have been established (Clifton and Clifton 1998). Therefore, it is unlikely that fishing pressure can explain decreased fish abundances on Cayos C.

In some studies, percent cover of live coral showed a significant positive correlation with fish species richness and abundance on shallow coral flats (Bell and Galzin 1984; Chabanet et al. 1997; Garpe and Ohman 2003). The current study, however, did not find a significant difference in live hard coral (average percent cover or species richness) between

islands or within sites. Therefore, this variable was not an important determinant of fish numbers.

However CCA ordination analysis shows that algae, which was significantly higher on Cayos C., and rugosity affect the nursery fish assemblages. In addition, results of the present study showed that the nursery species *Abudefduf saxatilis* was negatively correlated with algae on coral reefs. Further research must be implemented to study the negative correlation between *A. saxatilis* and algae.

Seven non-nursery species experienced higher abundances on Utila than Cayos C., which may be a result of these other explanatory variables. Regardless, it is important to emphasize that a larger percentage of nursery species had higher abundance on Utila than did non-nursery species. Therefore, comparisons between coral reefs off of mangrove-rich islands and mangrove-poor islands indicate that mangroves may increase the abundance of nursery coral-reef fish. This study adds one data point (island comparisons of fish nursery species) to a large scale graph of comparable matched-pairs from prior studies (four studies finding positive correlations between mangroves and adult coral reef fish and two showing no correlation (Halpern 2004, Nagelkerken 2007)) to give statistical power to comparing fish assemblages in the presence/absence of mangroves.

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Effect of hurricane John (2006) on the invertebrates associated with corals in Bahía de La Paz, Gulf of California

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Abstract. During the last four years, quarterly visual censuses were conducted on reef communities at six sites around Bahía de La Paz (La Paz bay) using belt transects (10 × 1 m, n = 5 replicates per site). The present study documents the effect of one hurricane on the reef invertebrate's assemblage found at these sites by comparing community structure and abundance before and after the passage of hurricane John (September 2006, level II Safir/Simpson—winds of 215 km/h). On July 2006 (prior to the hurricane), visual censuses revealed 50 species and 3093 specimens, whereas in October 2006 (after the hurricane) 39 species and 2018 specimens were found, with a similarity index of 83.1% between these two surveys. There were 13 species that were not seen after the storm and three species were registered as "new" after the hurricane. The group most affected was the Echinoderms, with six species lost. The sighting of new species was mainly for the crabs, with two species. One year after the hurricane (October 2007), the recovery of the community was 38% of richness, with the addition of five species (three Echinoderms and two Mollusks) that were lost after the hurricane.

Key words: Monitoring, Reefs, Succession, Visual censuses.

Introduction

Marine invertebrates are important components in benthic communities where, among other functions, play an essential role by transferring energy up to higher trophic levels. For the Gulf of California, México, numerous studies on invertebrates have been conducted (i.e., Brusca 1980; Wicksten 1983; Villalobos et al. 1989; Hendrickx 1993). However, most of these papers were prepared under a taxonomic perspective and based on collections and voucher specimens from museums.

Bahía de La Paz (located in the southern Gulf of California, near the city of the same name) is probably the best studied location in the Gulf of California because of the presence of several marine sciences institutes in the area (Solís-Marin et al. 1997; González-Medina et al. 2006). Systematic checklists of different taxonomic groups such as fish (Castro-Aguirre and Balart 1997), echinoderms (Solís-Marin et al. 1997), mollusks (Holguín-Quiñones and García-Domínguez 1997), and decapods (Pereyra 1998; Hernández 1999) are available for this area. Many of these studies were performed on coral areas, where researchers used destructive methodologies to obtain specimens. These procedures were "acceptable" in the past, but after the mass mortality of corals occurred because of the increase in water temperature brought upon by the El Niño Southern Oscillation (Reyes-Bonilla 2001), there is growing concern that destructive sampling can exacerbate the natural degradation of these fragile systems. To avoid this problem, descriptive studies are now conducted chiefly using visual census methods.

The southern Gulf of California (where La Paz is) suffers the frequent impacts of hurricanes and tropical storms, but there are few studies on the damage that these disturbances exert on marine communities, and especially on the coral reefs of this area. Only Reyes-Bonilla (1993, 2003) and Glynn (2001) have provided information on this topic and described how hurricanes have actually caused limited damage to the urchin and fish populations; moreover, the hurricanes sometimes have even benefited coral dispersal by an increase in fragmentation. Considering the dearth of available information, the objective of this paper was to illustrate changes to the invertebrates' assemblages in coral habitats after the passage of a strong hurricane. Our results show how the impacts were immediate and sustained, as one year after the perturbation, the assemblages had not returned to their original state.

Material and Methods

Bahía de La Paz is located in the southwestern Gulf of California between 24° 11´ to 24° 40´N, and 110° 20´ to 110° 42´W. On September 2006, hurricane John (level II Safir/Simpson—winds of 215 km/h) impacted the area (Fig. 1) and caused severe disturbance to the benthic communities along its path. In the present study, surveys were conducted in July 2006 (before the hurricane), October 2006 (after the hurricane), and one year after the event (October 2007), in order to measure the impact and potential recovery of invertebrate assemblages.

Surveys were conducted on coral reefs or communities (sensu Reyes-Bonilla 2003) at four sites using belt transects (10×1 m, n = five replicates per site). A SCUBA diver visually identified and counted the invertebrates seen inside the belts. The taxa surveyed included mollusks (Gastropoda), sea urchins (Echinoidea), sea stars (Asteroidea), decapod crustaceans (Anomura, Brachyura, and Caridea), and polychaetes (Annelida). From these records, several community descriptors (Magurran 2003) were calculated to compare the structure of the communities over time [similarity (Bray-Curtis coeff-

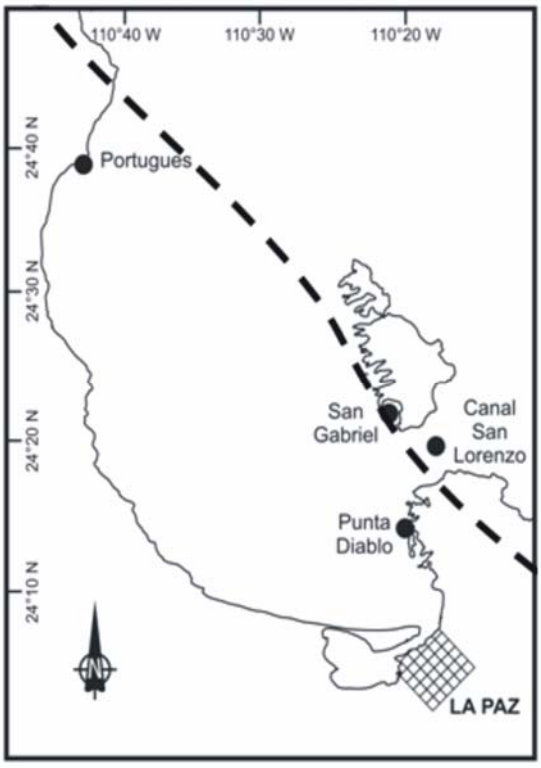

Figure 1: Study area (Bahía de La Paz) and survey sites. The dashed line shows the trajectory of hurricane John (August-September 2006).

icient), diversity (Shannon-Wiener index), and evenness (Pielou index)]. We used a non metric multidimensional scaling (Clarke and Gorley 2006) to map the degree of similarity among sampling sites (before, after, and one year after the hurricane). A cluster analysis was applied to view the similarities in species richness among sampling dates (July 06, October 06, and October 07). Comparisons among surveys at each site were done using 1 way ANOVA.

Results

Species richness and abundance of invertebrates decreased after the passage of the hurricane; the change was remarkable as these descriptors were down from 20% to 30% of initial (Table 1). In contrast, the diversity and evenness practically remained the same. To confirm that changes were caused by the hurricane and do not represent the seasonal variation, we plotted the annual density on July and October from 2005 to 2007 (Fig. 2). In the graphic is evident that the average density on October 2006 was lower than in 2005 and 2007 and the difference is statistically significant ($F= 4.94$, $p<0.05$). This found represents the hurricane effect on the invertebrates' assemblage.

Table 1. Community parameters of invertebrates' assemblage in La Paz bay before and after hurricane John (S= species richness; N= abundance, J´=evenness, H´= diversity).

	S	N	J´	H´
July 06 (Before)	50	3093	0.94	3.67
October 06 (After)	39	2018	0.96	3.51

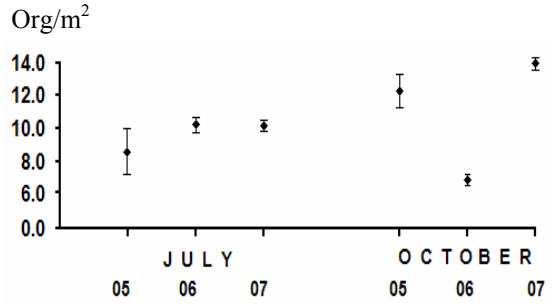

Figure 2: Density of invertebrates including the same months one year before and one after the hurricane, to show annual tendencies. (average ± standard deviation).

There were also changes in species composition. Fourteen species found before the hurricane (July 06) were not observed after the storm (October 06). The most sensitive group was the echinoderms with seven species lost (i.e. *Amphiaster insignis, Euapta godefroyi, Isostichopus fuscus*) followed by mollusks with five species (i.e. *Megapitaria squalida,*

Muricanthus nigritus, Nodipecten subnodosus), and crustaceans and annelids with one species each.

On the other hand, Crustaceans practically did not change in abundance from 897 individuals before the hurricane, to 908 just afterwards. Remarkably, their numbers rose to >2500 individuals one year after the perturbation. Within this group, the opportunistic, coral-symbiotic crustacean species were the most abundant (Table 2).

Table 2. Abundance of most sensitive species before and after hurricane John (July 06 vs. October 07). Groups: Cru =Crustaceans, Ech = Echinoderms, Mol =Mollusks. * = Symbiotic to pocilloporid corals.

Group	Species	Jul 06	Oct 07
Cru	*Alpheus lottini**	191	488
Cru	*Calcinus californiensis*	22	61
Ech	*Diadema mexicanum*	123	18
Ech	*Echinometra vanbrunti*	319	89
Cru	*Harpiliopsis depressa**	192	718
Cru	*Harpiliopsis spinigera**	80	507
Cru	*Neaxius vivesi*	58	2
Mol	*Quoyula madreporarum**	30	115
Cru	*Trapezia ferruginea**	251	538

A comparison of similarity among sites based on species composition and abundance was performed whit data taken just after de hurricane (October 06). The community at San Gabriel reef was very different from the rest, although this difference was not significant (stress > 0.10: Fig 3).

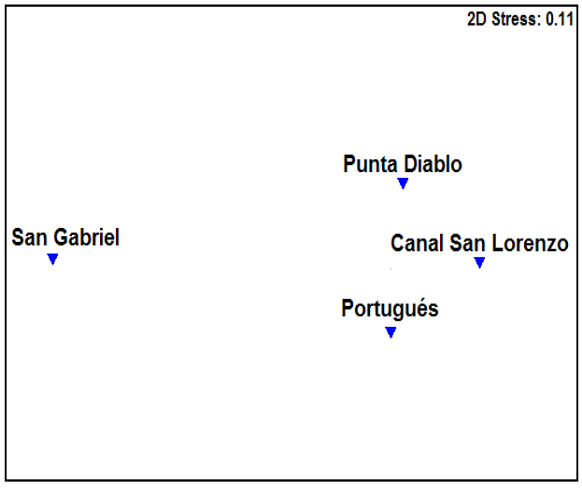

Figure 3: nMDS of invertebrates' abundance on the sampled sites.

Comparisons over time indicated that species richness after the disturbances (October 2006 and October 2007) were more similar between them, than those sampled prior to the storm (Fig. 4).

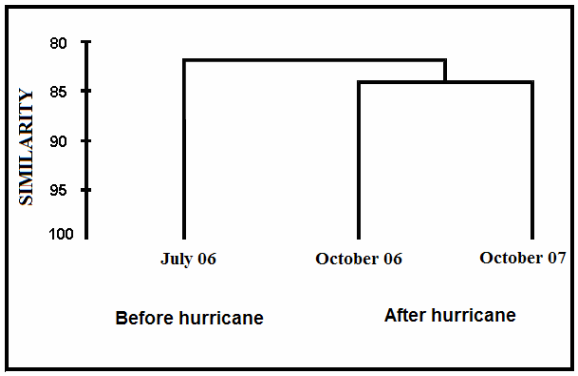

Figure 4: Similarity cluster (Bray-Curtis) on species richness of invertebrates from Bahía de La Paz before and after hurricane John.

Discussion

The present study showed that community parameters changed after the hurricane. Commonly, after any disturbance in this kind of ecosystems, dominance decreases and diversity increases (Rogers 2003). However, in the present study, we detected important changes in richness and abundance, whereas evenness and diversity remained at similar levels. The slight increase in equitability (Table 1) was maybe related to a more equal distribution of abundance among the species, caused by the change in the substrate heterogeneity promoted by the storm. However, in the present study diversity and evenness did not change significantly with time. As these markers are defined by an interaction of richness and relative abundances (Salazar-Vallejo 2002), we suggest that species replacements after the hurricane may have caused the detected stability.

The physical damage caused by hurricanes can be considerable. Lugo-Fernández et al. (1994) showed that waves were the main agent that caused changes in Margarita reefs (Puerto Rico). At San Gabriel, coral pieces broken by the hurricane covered the sandy-gravel bottom and, thus, caused some sedentary species to be removed, including the clams *Megapitaria squalida* and *Nodipecten subnodosus*. On the other hand, organisms that are usually not seen in visual surveys (i.e., the crab *Eriphia squamata*, the clam *Pinna rugosa*), were exposed by the broken corals after the hurricane (October 2006). Notwithstanding the higher probability of observing specimens, richness was still lower than before the storm (July 2006). Thus, the hurricane caused an immediate greater impact on species richness, and also qualitatively changed the community.

In addition, coral fragmentation caused by the hurricane at San Gabriel promoted an increase in availability of coral substrata and, as a consequence, there was an increase in abundance one year after the

storm compared to previous dates. Interestingly, most of the higher abundances were due to the arrival of coral-symbiotic species (Table 2).

One year after the hurricane John (October 2007), there were numerous echinoderm and mollusk recruits, which will likely recover baseline conditions in the reef areas visited. Rogers (2003) stated that the recovery of any community will depend, on the natural resilience of the species that compose the community. In the case of La Paz, there were no hurricanes of consideration in 2007 and 2008; if this trend continues, it is feasible that the conditions may improve in years to come. In the absence of storms, an increase in the abundance and richness concomitant with a less homogeneous community where the dominant taxa will regain their advantage would be expected.

In the present study, the before-after comparison was accomplished thanks to a monitoring program that was initiated in 2005 using methods that do not disturb the corals and associated fauna (Halford and Thompson 1994). Visual surveys present advantages over destructive techniques in the study of disturbed areas, as the corals suffer no further harm. Finally, the results presented here serve as a baseline inventory of coral-associated invertebrates for future evaluations. The data collected will also help local environmental authorities in the monitoring and management of protected areas, especially considering that San Gabriel reef is now part of a recently established National Park.

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Carry-over effects - the importance of a good start

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Abstract. Every organism has a history and its past experiences shape its present capabilities and may greatly influence the outcome of future events. For marine organisms with complex life cycles, seemingly discrete life stages separated by periods of rapid change (metamorphosis) have led researchers to focus on processes that occur within stages. Recent research highlights the interconnections among life stages and underscore that phases within a life cycle are functionally coupled. Success in one stage biases the probability of outcomes in subsequent stages. The present study reviews research on model species of tropical reef damselfish, *Pomacentrus amboinensis*, to highlight the fundamental importance of effects that 'carry-over' from previous life stages and potentially generations. Research strongly suggests that the physiological state of parents (particularly mothers) during gametogenesis pro-rate offspring success and may even influence their fitness.

Key words: parental effects, metamorphosis, coral reef fish, history, phenotypic selection

Introduction

The life cycles of marine organisms are comprsed of a series of discrete life stages. Transitions between stages differ in their nature and the magnitude of change and are species specific. Each life stage will have a different primary role (development, growth, recruitment, reproduction), and will be responsive to, and affected by, prevailing conditions differently. Because of the dramatic changes in function, physiology and environment that characterize sequential life stages it cannot be expected that advantages gained in one life stage (e.g. the ability to capture planktonic particles) will necessarily result in an advantage in the next stage. However, recent evidence suggests that the way individuals respond to conditions in one life stage can influence their characteristics in subsequent life stages. Individual characteristics of one particular life stage have been found to positively influence the next life stage, but they can also influence multiple life-stages and even may extend between generations (Green and McCormick 2005; Giménez 2006).

Because the intensity of selection on phenotype, life history or behavioural traits will change with ontogeny it is important to know the extent to which an individual's characteristics are influenced by its history and the extent to which its success is independent of past events.

How an individual's history influences present and future outcomes, now known as 'carry-over effects', has recently become a focus of interest for marine researchers. Researchers on invertebrates in particular have recently focused attention on the links between life stages (Podolsky and Morany 2006 and references in same issue), possibly because of the greater ease of laboratory rearing and relatively short longevities of invertebrates. Researchers on fishes have typically underplayed the importance of life history interconnections, despite a large body of research from a diverse range of fields that underscores their importance.

Our objective is to highlight the importance of carry-over effects to coral reef fishes by briefly reviewing research on one Indo-Pacific damselfish (*Pomacentrus amboinensis*) that has been used as a model for many different types of biological, ecological and evolutionary studies. Using these studies we emphasise the interconnection among life stages and the ramifications of these for population dynamics. The link between the quality and performance traits of fish and their numerical population consequences has been explored elsewhere (Jones and McCormick 2002).

Parental influences on larval traits

Recent research suggests that parental attributes of coral reef fishes greatly influence developmental and performance trajectories of larvae through the provisioning of the gametes during gametogenesis, as shown in other organisms (Mouseau and Fox 1998). Studies of *P. amboinensis* have emphasized the importance of maternal body condition and physiological state in influencing embryo and larval phenotype, with female

effects being stronger than male effects (Table 1). A study that monitored the reproductive output of 40 breeding pairs on 2x2m patch reefs found that females of smaller size that were in better than average body condition produced larvae that had larger nutritional stores (Table 1), while female age had little influence on larval attributes at hatching. When females were placed into three size categories it was evident that small females produced larvae that were both longer and had larger yolk-sacs at hatching (Table 1).

Table 1: The influence of female and male *P. amboinensis* attributes on larval morphology at hatching, from breeding pairs on 40 patch reefs in the Lizard Island lagoon, Great Barrier Reef (GRR). Significant partial correlations are given; ns indicates no significant relationship between the two variables at α=0.05. Condition of females (cond) was quantified as the residuals of a length/wet weight regression. Females ranged between 40.8 to 66.4mm standard length, and were 2 to 6 years old. n = 25 per comparison. Larvae were preserved and measured as per McCormick (2006) and aged following Maddams (2007). Larval attributes are for the second clutch produced. Source: Maddams and McCormick unpublished.data.

	Breeder attributes			
Larval traits	♀ size	♀ age	♀ cond	♂ size
Larval length	ns	ns	ns	ns
Yolk sac area	-0.65	ns	0.60	ns
Oil globule area	-0.60	ns	0.47	ns
Eye diameter	ns	ns	ns	ns
Head depth	ns	ns	-0.46	ns

Experiments that supplementary fed breeding pairs in the field have shown that maternal body condition influences the yolk-sac size of larvae at hatching, but has little influence on larval length (Kerrigan 1997; McCormick 2003; Fig. 1).

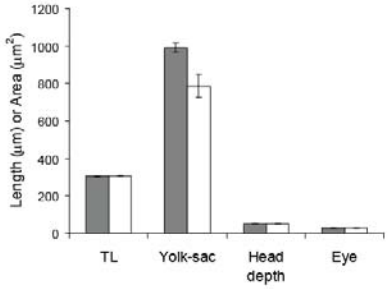

Figure 1: Comparison of morphologies of larvae at hatching from *P. amboinensis* breeding females that have (grey) or have not been supplementary fed (white bar) in the field for 5 min per day (from McCormick 2003). Errors are standard errors.

In contrast, parental interactions that alter maternal stress levels, such as density-dependent interactions with con- or hetero-specifics, directly influence larval length at hatching through a mechanism involving the stress hormone, cortisol (McCormick 1998, 1999, 2006, 2009; McCormick and Nechaev 2002). Experimental manipulations of maternal cortisol within naturally occurring limits were able to produce larvae that spanned the complete size range that naturally occurred in the wild, emphasizing the importance of this mechanism (McCormick 1999, 2006). Interestingly, laboratory manipulations of maternal testosterone were found to influence yolk-sac utilization rates.

Maternal stress has also been shown to influence the inter-relationships among early life-history traits in *P. amboinensis*. By manipulating the cortisol environment of individual embryos, Gagliano and McCormick (unpublished data) have been able to show that the fundamental relationship between early life history traits changes, emphasizing the potent role of the maternal state at gametogenesis for subsequent larval performance and survival (Fig. 2). Laboratory manipulations of cortisol and testosterone have previously been shown to influence the relationship between larval standard length at hatching and yolk sac area (McCormick 1999).

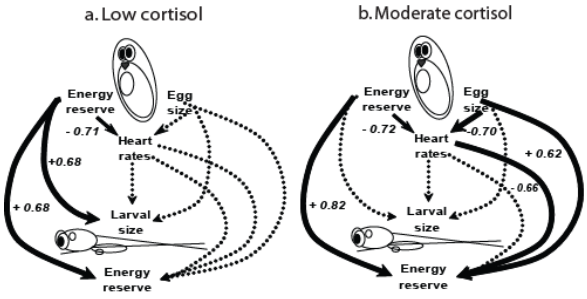

Figure 2: Summary of the correlations among early life history attributes of *P. amboinensis* embryos at 36 h post-fertilization (pf) with heart rate at 84 h pf and attributes of newly hatched larvae. Correlations for embryos incubated with no additional cortisol added to seawater (a) are compared to embryos incubated in seawater supplemented with a cortisol solution (10^2 ng/ml) to mimic moderate levels of maternal interactions (b). Protocols followed McCormick (1999) and Gagliano et al. (2007b). n=72. Bold lines represent significant relationships.

Similar laboratory manipulations have indicated that aspects of the sensory system are also influenced by maternal stress. Recent experiments found that maternal stress directly influenced the fluctuating asymmetry (FA) of the sagittal otoliths (Gagliano and McCormick submitted). A field experiment showing the relationship between otolith asymmetry and attraction to specific sounds suggests that FA may be important in navigation (Gagliano et al. 2008). There is also the indication from field data on *P. amboinensis* (Gagliano and McCormick submitted) and on a tropical lizardfish (*Saurida suspicio*) (Lemberget and McCormick 2009) that larval survival is directly and strongly related to the degree of otolith FA. These studies directly link larval survival and cohort strength to maternal influences.

Initial characteristics influence larval and juvenile survival

The ubiquitous problem of collecting identifiable larva at anything other than the start or end of their larval stage has made studying the processes that influence survival difficult for tropical marine organisms. This has meant that much of the research conducted on fish larvae to date has examined the Olympians that survived the larval phase. From these we attempt to make inferences on the processes that facilitated their survival, and what influenced those that died.

In the most comprehensive study to date, Gagliano et al. (2007a) serially sampled a pulse of *P. amboinensis* from production through to 2 months post-settlement (ps) and examined phenotypic selection using the history of growth stored in otoliths. They found strong links between early life history traits and persistence, with even the earliest traits, such as size of the sagittal otolith at hatching, still being selected for well into the juvenile phase (Table 2). Of the diverse traits measured, growth during the larval phase was the trait with the longest duration of influence, with selection still being detectable 6 weeks into the juvenile phase (Table 2).

Table 2: Carry-over effects of (a) phenotypic traits, as represented by otolith size, and (b) otolith growth on subsequent survival from serial samples of a reproductive pulse of *P. amboinensis* from Lizard Island, GBR. Summary indicates the duration that selection on various attributes is detectable and the direction of that selection. ns, no significant selection. (*after* Gagliano et al. 2007a).

Attribute selected	Detectable to:	Direction
a. Phenotype		
Hatch	2 wks ps	-
Settlement	4 wks ps	-
2-wks ps	ns	
3 wks ps	4 wks ps	-
4 wks ps	ns	
b. Growth period		
Larval phase	6 wks ps	-
First 2 wks ps	3 wks ps	+
Third wk ps	4 wks ps	-
Forth wk ps	ns	

Field and laboratory experiments have shown that settlement represents a critical phase for *P. amboinensis* with mortality showing a Type III trajectory and being highly selective for a variety of traits (McCormick and Hoey 2004). Overall, fish that were larger at settlement, that had larger lipid stores and who had higher larval growth, had a survival advantage (Table 3). The attributes that influence *P. amboinensis* survival at settlement are, however, dependent upon the location they settle into and the resident community at that location (Holmes and McCormick 2006).

A field experiment by McCormick and Meekan (2007) showed the characteristics that influence prey survival were dependent upon the patch-work of predator selection-fields into which the naïve juveniles settled. Juveniles that settled into nesting

Table 3: Summary of the selectivity of mortality on various phenotypic attributes for newly settled *P. amboinensis* that were naïve to predators. A positive selection direction means that individuals that had a larger value on that trait had a higher probability of mortality. In the laboratory trials the dottyback, *Pseudochromis fuscus*, was used. Laboratory and field trials with weight and burst speed were size standardized. ns, non-significant effect. FA: fluctuating asymmetry. References: 1, Gagliano et al. 2007a; 2, Gagliano and McCormick 2007; 3, Hoey and McCormick 2004; 4, McCormick and Hoey 2004; 5, Holmes and McCormick 2009; 6, Gagliano et al. 2008.

Phenotypic trait	Field (F) or Lab(L)	Selection direction	Reference
Otolith size at hatching	F	-	1

Otolith size at settlement	F	-	1,
Size at settlement	F	-	2, 3, 4
	L	-	5
Weight at settlement	FandL	+/ns	5
Lipid levels at settlement	F	-	3
Burst speed at settlement	FandL	ns/ns	5
Fulton's condition factor	F	+	3
Larval growth	F	-	1, 3
Otolith FA at settlement	F	+	6

territories of male *P. amboinensis* were exposed to positive selection by the dottyback *Pseudochromis fuscus*, which prefers large recruits and who were not evicted by aggressive male territory holders. Outside the territories, where the juveniles were exposed to a broader range of predators, larger individuals had higher survival.

Initial larval characteristics influence individual fitness

Recently, studies of marine organisms have found a link between early life history traits and adult success. Many tropical fishes exhibit complex ontogenies involving sex change. The most common form is protogyny, where juveniles first mature as females and then undergo sex change in the event of the appropriate social trigger (monandric protogynous hermaphrodism; Munday et al. 2006). In these species, individuals that attain the status of male get to monopolise matings and have the highest fitness. *P. amboinensis* is a damselfish that exhibits protogynous hermaphrodism. Male and dominant female *P. amboinensis* were collected from around Lizard Island and growth histories were reconstructed using otolith increment widths from cross sections of the sagitta as a proxy for growth. For a portion of these fish (up to 7 years old) it was possible to obtain measurements from the larval otolith. Those females that had undergone sex change and became males were found to have significantly smaller increment widths compared to those that were still female ($t = 2.32$, df 11, $p = 0.04$; Fig. 3). Of the six other species from 2 families that we have examined, 3 have shown similar relationships, with significant differences in larval growth starting with the hatch mark, between females and those fish that become males later in life (1 out of 2 sandperches, 2 out of 4 wrasse; Walker et al. 2007; McCormick and Ryen unpublished data).

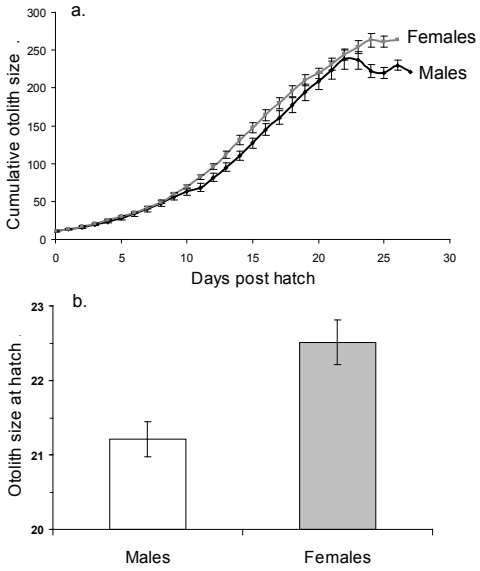

Figure 3: Early otolith growth of females and females that have changed sex to become males showing cumulative otolith growth through the larval phase (a), and differences in increment widths between sexes at hatching (b). Errors are standard errors (n = 8 females and 5 males).

Transgenerational effects

The pelagic larval phase of almost all coral reef fishes makes it difficult to track the fate of individuals from when first produced from parents of known history through to becoming an old member of a reproductive group (i.e. one with measurable fitness). While transgenerational studies are presently being conducted on a brooding

species (*Acanthochromis polyacanthus*; e.g. Donelson et al. 2008), the rarity of the strategy to have non-dispersive larvae means that results, whilst useful, will have limited applicability to the population dynamics of most marine fishes. We must therefore make the best of inferences we can make on the connections between generations.

Otoliths are a valuable record of relative growth and may prove a useful tool in linking parental influences to the growth dynamics of their offspring. Unfortunately, while we know that the relationship between somatic growth and otolith growth can be individualistic (Campana and Jones 1992), we have little understanding of the plasticity of the relationship, the factors that will influence it and the potential lag effects for most life stages of fish (see Molony and Choat 1990 for juveniles). A recent study that has simulated the effects of maternal stress on the embryogenesis of individual *P. amboinensis* found that maternal cortisol levels not only influenced the strength of the relationship between early life history traits, but influenced the fundamental relationships between phenotypic traits and otolith size at hatching (Table 4). Under a low stress scenario, otolith size at hatching was negatively related to initial and hatching yolk size and to heart rate (at 84h post-fertilization; a proxy for metabolism). This is opposite to the relationship found for 7 juvenile freshwater pike, where metabolism was positively associated with the width of increments deposited (Armstrong et al. 2004). Interestingly, when simulated maternal stress increased, the relationships between early life history attributes changed. It was only under moderate levels of stress that a positive relationship was found between otolith size at hatching and larval length (Table 4). It is this positive relationship between otolith size and larval size that is often assumed to be present in previous studies (Vigliola and Meekan 2002). Clearly further work is required to determine the drivers of increment width.

Table 4: Relationship of otolith (sagitta) size at hatching with early life history attributes for *P. amboinensis* embryos individually reared from fertilized eggs under three simulated maternal stress levels. Low stress (no addition of cortisol, equivalent to parents being on a patch reef alone); Moderate stress (addition of low cortisol dose, equivalent to the mother interacting with 2 conspecifics on a patch reef); High stress (high cortisol dose, equivalent to the mother interacting with 4 to 6 other conspecifics on a patch reef). For detailed methods see Gagliano and McCormick (submitted).

	Otolith size at hatch versus			
Treatment	Yolk size of egg	Heart rate	Yolk size at hatch	Length at hatch
Low cortisol	-0.71	-0.78	-0.69	ns
Mod cortisol	ns	ns	ns	+0.63
High cortisol	-0.88	ns	ns	ns

While the exact meaning of the otolith size at hatching is presently unclear, evidence suggests that maternal effects are likely to have a major influence on the relationship between early life history traits and otolith characteristics. The relationship between initial otolith size and fitness, together with the influence of maternal stress on larval size and otolith FA (McCormick 2006; Gagliano and McCormick submitted), suggest that maternal characteristics may play an important role in predetermining the subsequent fitness of individuals; the ultimate carry-over effect.

Carry-over effects have been documented in many marine phyla. There are many studies of fish that have shown that previous history influence future success (Berkely et al. 2004; Sponaugle and Grorud-Colvert 2006). These links occur between adjacent life-stages, across multiple stages and across generations. To date, few marine studies have managed to demonstrate transgeneration effects because of the logistical difficulties in linking all the stages in organisms with complex life cycles. Available data, such as that compiled here for one model fish species, suggests that transgenerational effects may be very important in influencing population dynamics. The key is to focus attention on how an individual's history influences present and future outcomes. The recent findings of high levels of self replenishment on small spatial scales for reef fishes with substantial larval durations (Almany et al. 2007) opens up additional possibilities for rapid local scale adaptation to changing conditions through maternal effects. The strong physiological, developmental, behavioural and performance links between stages and potentially between generations, mean that carry-over effects are fundamental forces that have been important in the shaping of existing populations and communities. The challenge is to define the extent to which carry-over effects influence present day dynamics and their importance in ecological and evolutionary time scales.

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Reef Communities in the Dry Tortugas (Florida, USA): Baseline Surveys for the New No-take Area

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Abstract. To understand the current community structure on reefs in the Dry Tortugas, we conducted species-level surveys of macroalgae, coral diversity, herbivorous and game fishes, urchins, and substratum composition (e.g., rugosity) in shallow (3- to 5-m depth) low-relief reef and hardbottom habitats in October 2007. We had particular interest in the ecological process of herbivory inside and outside of the "no-take" Research Natural Area (RNA) designated by the U.S. National Park Service in 2007, and establishing a baseline to assess future changes to trophic functioning. *Diadema antillarum* and herbivorous fish abundance, percent cover of macroalgae, and species richness of corals and gorgonians at the 18 randomly selected survey sites were not significantly different inside vs. outside of the RNA. Mean densities of *D. antillarum* ranged from 0.01 to 0.54 individuals m^{-2}, with 11 of the 18 sites having densities above 0.10 individuals m^{-2}. Both *D. antillarum* density and coral species richness were positively correlated to rugosity of the substratum. *Diadema antillarum* density was also positively related to percentage of the substratum composed of *Acropora cervicornis* rubble. Improved trophic functioning and increases in *D. antillarum* can improve reef condition in the Dry Tortugas, and the RNA is an important management tool to achieve increases in reef resilience to global-scale stressors.

Key words: community structure, urchins, herbivory, marine protected areas, marine reserves, *Diadema*

Introduction

The Dry Tortugas is a unique and isolated atoll-like reef system at the terminus of the Florida Keys. Like other reefs in the Florida/Caribbean region, this area has experienced significant community shifts indicative of degradation, with reduced live coral coverage, increases in macroalgae, and decreases in species targeted in fisheries. However, both local and global stressors probably contributed to these community shifts, and meteorological events are known to have impacted the reefs. Reef research has a fairly long history in the Dry Tortugas, beginning before the establishment of the Carnegie Institute Tortugas Laboratory on Loggerhead Key in 1905 (Davis 1982; Shinn and Jaap 2005). Alexander Agassiz mapped the Dry Tortugas benthos in 1881 (Davis 1982), showing tens of hectares of *Acropora palmata*, but coring has revealed that this species was not responsible for reef accretion in the Dry Tortugas (Shinn et al. 1977). In 1976, a comprehensive habitat map was created to examine community change since the Agassiz map, showing that the *A. palmata* had all but disappeared and that *A. cervicornis* coverage was vast (Davis 1982). In January 1977, a severe cold front killed most of the *A. cervicornis* reefs in the Dry Tortugas (Davis 1982; Porter et al. 1982). Populations have not recovered on patch reefs once dominated by this species in the early 1970s; in fact, a representative site showed continued decline from 1999 to 2005 due to bleaching and disease (Beaver et al. 2005).

The purpose of our study was to characterize the current biological communities within Dry Tortugas National Park to provide important baseline data for assessing the effects of the "no-take" Research Natural Area (RNA) designated by the U.S. National Park Service (NPS) in 2007. We also sought to document any recovery of populations impacted during documented disturbances (Porter et al. 1982; Beaver et al. 2005), and to assess the ecological process of herbivory inside and outside of the RNA prior to any RNA-induced changes to trophic structure that may occur due to the new management regime. Because of our interest in herbivory, and because most other monitoring programs (e.g., NPS-South Florida/Caribbean Inventory and Monitoring Network, National Oceanic and Atmospheric Administration, NPS-DTNP and Florida Fish and Wildlife Research Institute) have focused on habitat > 6 m, we chose to focus our study on the shallow (< 5 m) reef environment. Recovery rates of the benthic community can increase within well-enforced marine protected areas (Mumby et al. 2006; Hughes et al. 2007); our long-term goal is to test this hypothesis.

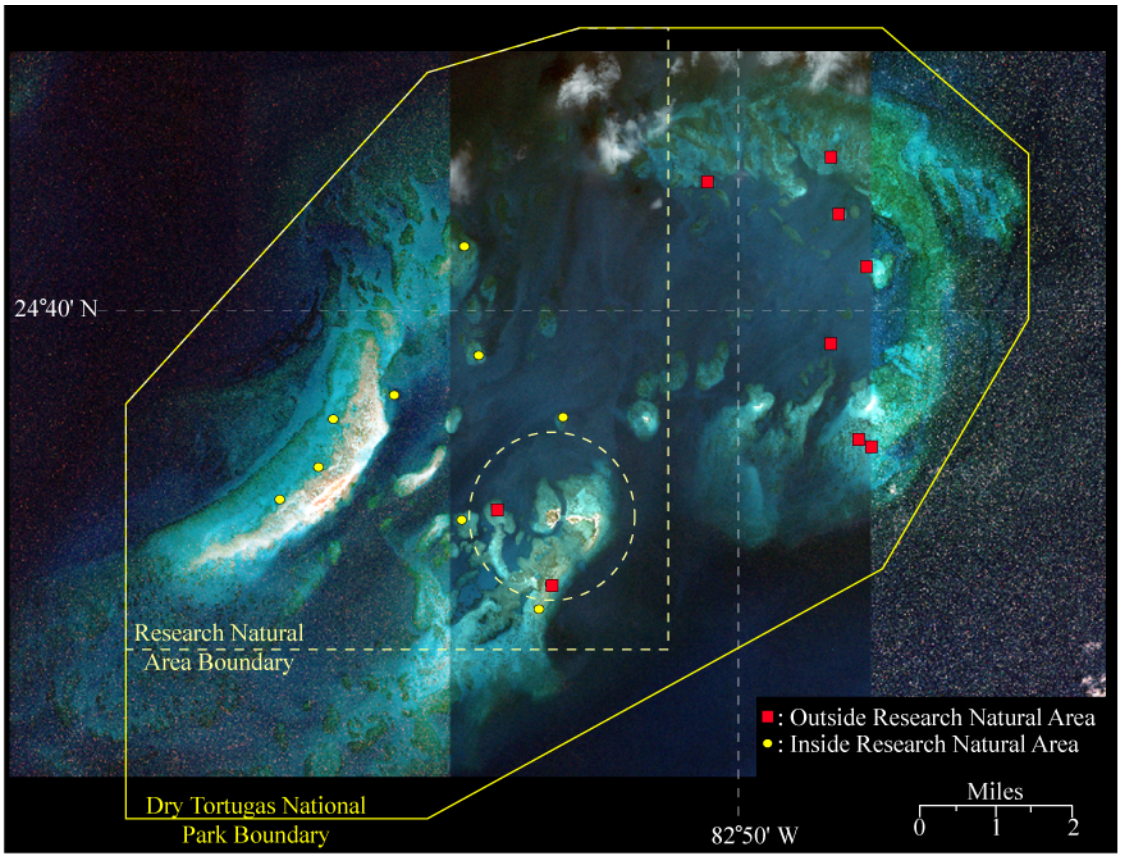

Figure 1: IKONOS satellite mosaic of Dry Tortugas National Park with the locations of 18 randomly selected sites where *in-situ* surveys were conducted October 16 – 20, 2007. The "no-take" Research Natural Area is within the dashed lines in the NW quadrant of the park and does not include the area within the circle.

Material and Methods

We randomly selected 18 sites for surveys, nine inside and nine outside of the RNA (Fig. 1), all within shallow (3- to 6-m depth) low-relief reef and hardbottom habitat. We conducted species-level surveys of macroalgae, scleractinian and gorgonian corals, herbivorous and game fishes, urchins, and substratum composition (e.g., rugosity). Three 15-m transects were laid out radially from each GPS location at bearings of 0°, 120°, and 240°. One Bohnsack-Bannerot visual fish census (7.5-m radius cylinder with stationary observer) was conducted on each transect line (Bohnsack and Bannerot 1986). The benthic community was assessed at n = 4 quadrats (0.0625 m^2 with line demarking 25 equal boxes) per transect by visually estimating percent cover of taxa within the quadrat, and urchins and damselfish were counted in a 2-m-wide belt along each transect line. Rugosity was estimated by draping a chain (3-cm link size) on the substratum underneath each transect line, giving the ratio between contoured and linear distance. Gorgonian and scleractinian diversity at each site was determined by recording all species observed throughout the approximately one-hour-long dive in the proximity of the three transect lines (≈700 m^2 area). The "game fish" category used here included grouper (*Epinephelus*, *Mycteroperca*, *Cephalopholis*, but excluded the small-bodied *Hypoplectrus*), jacks (*Caranx*), snapper (*Lutjanus* and *Ocyurus*), and hogfish (*Lachnolaimus maximus*). Biomass of herbivores was calculated by using length to weight conversions $W=aL^b$, where W = weight in g, L = fork or total length (species dependent) in cm, and a and b are species-specific constants reported by www.fishbase.org. Total length was estimated during visual surveys and then converted to fork length when necessary using regression relationships published on www.fishbase.org.

Results

Our initial assessment of the 18 randomly selected shallow low-relief hardbottom sites revealed that no variables measured were significantly different inside vs. outside of the newly established Research Natural Area (Table 1). *Diadema antillarum* was present and fairly evenly distributed at all 18 sites. Mean densities ranged from 0.01 to 0.54 individuals m^{-2}, with 11 of the 18 sites having densities above 0.10 individuals m^{-2}.

Table 1. Variables measured during *in-situ* surveys October 16 – 20, 2007, in Dry Tortugas National Park. Means, standard errors, and p-values of two-tailed two-sample t-tests are reported for variables comparing sites inside (n = 9) and outside (n = 9) of the "no-take" Research Natural Area established in January 2007. The sites outside are still within the National Park boundaries where there are limited restrictions on resource use, including a ban on spear fishing. "Encrusting invertebrates" includes only *Briareum asbestinum*, *Erythropodium caribaeorum*, and *Palythoa caribaeorum*. [a] = T-test assuming unequal variance using Satterthwait's method as reported by Statistix© 9 software.

Variable	Inside RNA		Outside RNA		T-test
	mean	SE	mean	SE	p =
Rugosity	1.33	0.04	1.29	0.03	0.49
No. of coral species	14.1	0.9	12.2	1.0	0.19
No. of gorgonian taxa	13.3	1.0	14.9	0.8	0.23
Diadema antillarum (no. m^{-2})	0.18	0.05	0.16	0.06	0.79
Acanthurids (no. $cylinder^{-1}$)	4.4	0.85	2.4	0.72	0.09
Acanthurid biomass (g $cylinder^{-1}$)	151	34	143	85	[a]0.93
Scarids (no. $cylinder^{-1}$)	14.4	3.2	13.9	2.9	0.91
Scarid biomass (g $cylinder^{-1}$)	418	105	283	152	0.48
Stegastes spp. (no. m^{-2})	0.66	0.04	0.7	0.13	[a]0.74
Game fish (no. $cylinder^{-1}$)	5.3	1.6	5.2	1.9	0.96
Crustose coralline algae (% cover)	33.9	4.7	24.8	2.9	0.12
Total calcified macroalgae (% cover)	9.0	1.1	9.0	0.44	[a]1.00
Total fleshy macroalgae (% cover)	32.9	3.8	34.3	2.6	0.80
Halimeda tuna (% cover)	6.2	1.0	6.8	0.5	[a]0.59
Dictyota spp. (% cover)	25.1	3.0	23.0	3.6	0.66
Encrusting invertebrates (% cover)	3.6	1.0	5.5	1.2	0.26

Table 2. Linear regression analysis of variables measured during *in-situ* surveys October 16 – 20, 2007 in Dry Tortugas National Park.

Independent variable	Dependent variable	Least squares linear regression		
		Transformation	R^2	p-value
Rugosity	*Diadema antillarum* density	Ln	0.31	0.017
Rugosity	Coral species richness	none	0.33	0.012
Rugosity	Scarid abundance	none	0.28	0.024
Rugosity	Scarid biomass	4^{th}-root	0.49	0.001
Rugosity	*Stegastes* spp.	4^{th}-root	0.29	0.021
Rubble (% cover)	*Diadema antillarum* density	none	0.36	0.009

Percent of the substratum covered by macroalgae was 34% and dominated by the brown algae *Dictyota* spp. *Diadema antillarum* density (Fig. 2A), scleractinian-coral species richness, scarid (parrotfish) abundance (Fig. 2B) and biomass, and *Stegastes* spp. (damselfish) abundance were positively related to rugosity of the substratum (Table 2). *Diadema antillarum* density was also positively related to percentage of the substratum composed of *Acropora cervicornis* rubble, and the amount of variance explained increased (adjusted $R^2 = 0.49$) when both the rugosity and rubble variables were included in a multiple linear regression model. Small (< 1-m-diameter), live colonies of *A. cervicornis* were noted at 12 of the 18 sites.

Discussion

The key question with respect to reef-resource conservation in the Dry Tortugas National Park is "Can we expect to reverse the decline in live coral coverage through the establishment and enforcement of the new no-take RNA?" To have realistic expectations for the RNA efficacy with respect to benthic communities, initial conditions must be assessed and forces that drive coral decline today and in the past must be understood. While there are too few ecological data to determine if the Caribbean-wide mortality of the key herbivore, *Diadema antillarum*, played a role in the replacement of live coral by macroalgae at this location since the early 1980s, it is reasonable to assume that similar changes in algal abundance documented after the die-off in other areas of the Caribbean (Morrison 1988; Carpenter 1990) also occurred in this location. Fishing pressure on herbivorous fishes is certainly a possible confounding factor in comparing sites within the Caribbean region, because scarids and acanthurids are not targeted in the local fisheries throughout the Florida Keys (Harper et al. 2000) whereas they are heavily targeted in places like Jamaica where fishing

pressure is especially intense (Hawkins and Roberts 2004). However, the grand mean of 24% coverage of the benthos by *Dictyota* spp. observed in this study indicates that the herbivorous fish population found in these hardbottom habitats is not sufficient to crop algae to levels documented prior to the 1980s, when benthic algae were thought of as "an inconspicuous component of coral reef environments" (Dahl 1974).

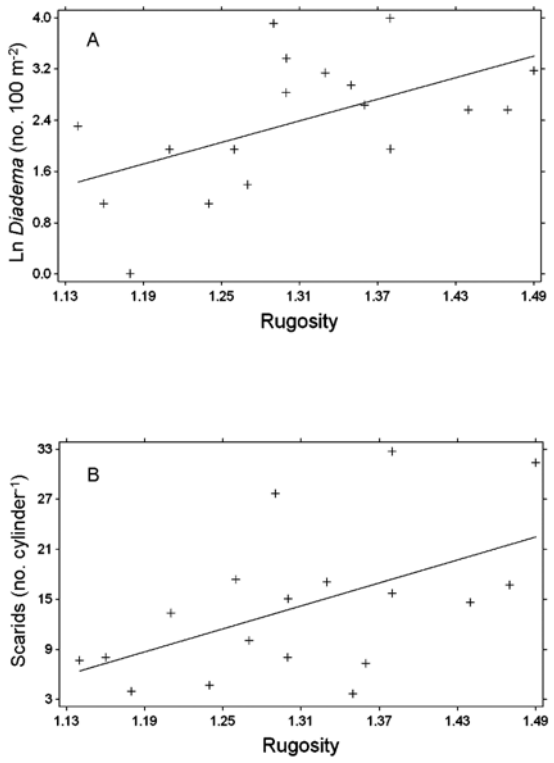

Figure 2: Linear-regression plots of A) *Diadema antillarum* density (Ln transformed data) and B) scarid (parrotfish) abundance (no. cylinder^{-1}) vs. substratum rugosity at 18 sites within Dry Tortugas National Park.

Recent increases in the *Diadema* population (Morrison, pers. comm.) could potentially enhance coral recruitment through the removal of *Dictyota* spp. and other algae from otherwise suitable substratum (Kuffner et al. 2006). Increased abundance of coral recruits in areas where *Diadema* density has rebounded has been documented on Jamaican reefs (Edmunds and Carpenter 2001). The fairly even distribution of low densities of *Diadema* observed in the Dry Tortugas is encouraging, since one of the main factors thought to hinder the recovery of this species is low-fertilization success in remnant populations due to long distances between reproductive individuals, i.e., the "allee" effect (Knowlton 2001).

There is no doubt that increased herbivory could help foster coral recovery. In this vein, the RNA could potentially contribute to restoring a coral-dominated benthic-community structure. However, the removal of stressors that have caused coral mortality in the past is also necessary to reverse reef degradation. As adults, corals may be superior competitors for space compared to most macroalgae, but once coral mortality occurs, the competitive hierarchy changes in favor of selected species of weedy primary producers (McCook et al. 2001; Kuffner and Paul 2004; Kuffner et al. 2006). Coral bleaching and disease are documented causes of recent coral mortality in the Dry Tortugas (Beaver et al. 2005), and since these are global- and regional-scale stressors, respectively, it is not likely that the newly established RNA will be effective in reducing coral mortality; however, managing for resilience could improve coral population recovery rates.

The positive correlation between *D. antillarum* density and percentage of the substratum composed of rubble revealed in this study indicates that testing the hypothesis that *A. cervicornis* rubble acts as nursery habitat for *D. antillarum* would be worthwhile. In an unpublished survey in St. John, USVI, juvenile (< 1 cm-diameter test) *Diadema* were only observed in rubble fields of similar grain size as in this study (Kuffner, unpublished). Chiappone et al. (2001) also noted in June 2000 that the two sites in the Dry Tortugas where they observed the highest densities of *D. antillarum* had large patches of *A. cervicornis* rubble. Little is known about the habitat requirements for recruitment in this species, most likely due to the rarity of finding individuals of < 1-cm-diameter test size and their cryptic juvenile behavior (Bak 1985).

It is too early to speculate whether the RNA will play a constructive role in the future of the benthic community in Dry Tortugas National Park. There is reason for optimism given the recent findings that well-enforced no-take reserves can have restorative effects on trophic functioning (Mumby et al. 2006). Mumby et al. (2006) demonstrated that levels of herbivory increased within a reserve compared to outside, resulting in significant changes to benthic community structure in the form of reduced levels of macroalgae. No-take areas are considered a key tool in managing for reef resilience (Bellwood et al. 2004; Mumby et al. 2007), and there is a growing body of evidence that no-take areas facilitate the recovery of key ecological processes like coral recruitment and herbivory (Hughes et al. 2007). As enforcement of the new management regime gets underway, the Dry Tortugas NP presents a unique case study of no-take area efficacy with minimal underlying problems of land-based pollution found in the adjacent inhabited Florida Keys.

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Physiological performance of giant clams (Tridacna spec.) in a recirculation system

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Abstract. The importance of auto- and heterotrophy for growth, survival, zooxanthellae development and nutrient uptake of juvenile giant clams *Tridacna maxima* and *T. squamosa* was investigated. In addition experiments on the photosynthesis, metabolism and calcification were performed with specimens of *T. maxima* and *T. derasa* in an intermittent flow-through respiration system. Animals were reared in a recirculation system under different light conditions and different concentrations of nutrients. Both particulate organic (*Tetraselmis* algae) and dissolved inorganic (ammonia and phosphate) food was given. Results show that light intensity and spectra have a significant effect on survival and length or weight increase. Nutrient concentrations only seem to play a role when clams are kept in optimum light conditions. In this case fertilization with NH_4^+ and PO_4^{3-} is more effective than feeding with algae. However, the concentration of zooxanthellae is only moderately increasing with increasing nutrient concentration. Moreover photosynthesis, respiration rates and calcification seem to be linked to light conditions but not to increasing nutrient concentrations. It is concluded that juvenile clams depend more on autotrophy to satisfy nutritional requirements. The uptake of nutrients is limited and needs to be tested with larger clams and a variety of nutrient combinations.

Key words: giant clam, nutrient uptake, growth, photosynthesis, respiration

Introduction

Giant clams are the largest bivalved animals in evolutionary history (Yonge 1975). They are effective filter feeders and at the same time autotrophic due to their symbiosis with zooxanthellae. This gives them a nutritional and growth advantage over normal heterotrophic bivalves. The filter feeding still remains a significant component of their procurement of nutrition (Klumpp et al. 1992). Although Klumpp and Lucas (1994) showed that photosynthates can contribute more daily carbon than actually needed, filter feeding seems necessary to satisfy the requirements for essential amino acids, phosphorous and trace metals (Hawkins and Klumpp 1995).

Although recent research into ecological and physiological background of different stages of the life cycle of clams made commercial aquaculture possible, there is still a high mortality with early stages. While juveniles rely on both heterotrophic and autotrophic feeding, adult clams depend more on available light. Particularly in aquaria the quality of the light plays an important role (Knop 1994) both with regard to light intensity and wavelengths, but negative effects of insufficient light sources are not known in detail.

Clams are raised commercially for human consumption in the Pacific region, sometimes also to replenish natural stocks (Lucas 1994). In addition they are a highly valuable resource for the international aquarium trade. However, losses during transport or during acclimation to aquaria are still high. Even after acclimation periods of several months, spontaneous mass mortalities in aquaria are reported (Knop 1994), most likely linked with water quality in closed recirculation systems. The availability of selected nutrients, particularly for the shell production, plays a key role.

Giant clams are known to prefer locations with gentle water movement, both for the supply of oxygen and nutrients (Knop 1994). When kept in high densities in small aquaria they are able to deplete nitrate and phosphate values considerably. This is why clams can play a potential role for bio-cleaning as additional water treatment module in a closed recirculation system.

Therefore, the importance of auto- and heterotrophy for growth, survival, zooxanthellae development and nutrient uptake of juvenile giant clams was investigated. In addition experiments on photosynthesis, metabolism and calcification under different light and nutrient conditions were performed.

The aims of this study are to:
1) estimate nutrient uptake rates for NH_4^+, PO_4^{3-} and food algae, 2) find optimal light conditions for growth, survival, uptake rates, 3) get an estimation of oxygen

consumption and photosynthetic rates and finally 4) get a first impression of zooxanthellae development and ETR efficiencies.

Material and Methods

From September 2006 to July 2008, 120 juvenile giant clams (*Tridacna maxima*, *T. squamosa* and *T. derasa*; Fig. 1) were reared in a closed recirculation system in ZMT Bremen (500 l volume, 32 PSU salinity, 27 °C temperature, 12 hours light vs. 12 hours dark regime, use of protein skimmer, calcium reactor, nitrate reactor, biofilter, UV units for water treatment). Maintenance conditions and species identification were performed after Lucas (1994) and Knop (1994).

Uptake rate and growth experiments with 30 (5-10 cm) specimens of *T. maxima* and *T. squamosa* were carried out for 21 days under different light conditions (use of HQI 150 W, HQL 125 W, fluorescent lamps 54 W, brand: Aqualine, actinic light) and different concentrations of particulate organic and dissolved inorganic nutrients (microalgae, NH_4^+ and PO_4^{3-}). For experiments (up to four hours exposure) elevated concentrations through spiking were used (5000 cells ml-¹ *Tetraselmis subcordiformis*; 0-150 µM NH_4^+, using ammonium sulphate; 0-10 µM PO_4^{3-} using potassium phosphate). Nutrients were measured with photometer after Koroleff and Grasshoff (1983); cells were counted with a Neubauer chamber. Length (mm) and weight (g) measurements were repeated every 7 days with calliper and electronic scale (Sartorius Germany).

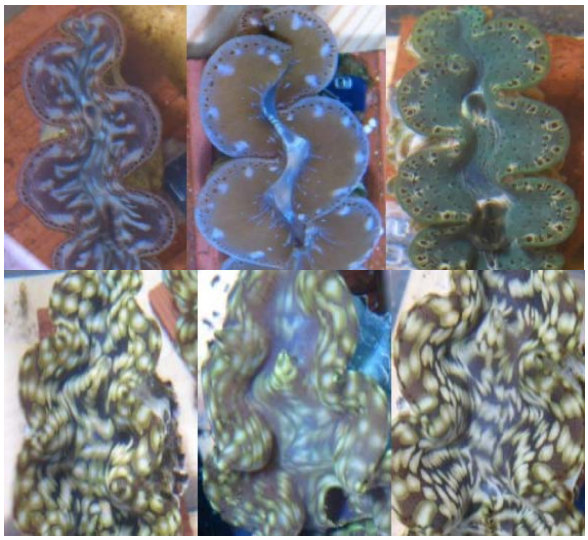

Figure 1: Juveniles of *Tridacna maxima* and *T. squamosa* in recirculation system in ZMT.

Experiments on photosynthesis (PS) and oxygen consumption (OC) were performed with 80 (5-8 cm) specimens of *T. maxima* and ten (10-12 cm) specimens of *T. derasa*.

A separate, PC-controlled intermittent flow respirometer with circular, flat-bottom acrylic respiration chambers of different volumes, according to size of animal, was used (detailed method see Kunzmann et al. 2007). 20 individual clams were measured for 2 hours in light and 2 hours in darkness. WTW 340i oxygen, pH and conductivity sensors were used for measurements of oxygen, salinity and temperature.

For PS measurements 20 individual clams and a PAM 2100 of Waltz (Germany) were used. Light measurements were performed with a Ramses ACC sensor from Trios (Germany), and a LI-250A light meter plus Hamamatsu Photonic multi channel analyser.

Results

Giant clams are capable of fast and efficient NH_4^+ (50 µM) and PO_4^{3-} (2 µM) uptake (Fig. 2, Fig. 3). Within less than 2-3 hours, nutrient concentrations elevated through spiking, are depleted down to normal levels. Only in the case of phosphate uptake in *T. squamosa* a slight delay was observed.

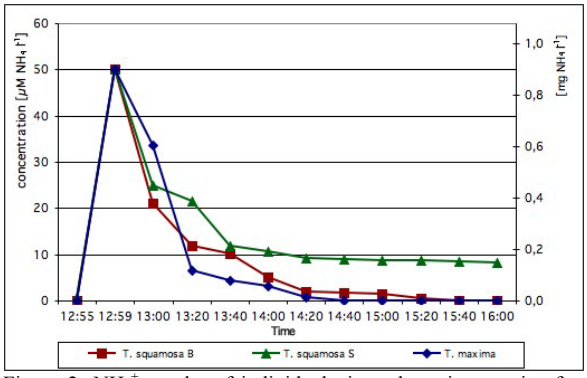

Figure 2: NH_4^+ uptake of individual giant clams in aquaria after spiking with 50 µM NH_4^+, using ammonium sulphate (n=1).

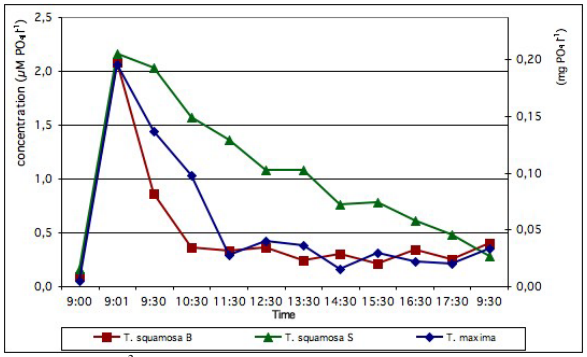

Figure 3: PO_4^{3-} uptake of individual giant clams in aquaria after spiking, using potassium phosphate (n=1).

Consumption of food algae cells reaches up to 7000 $g^{-1}h^{-1}$. The uptake rate was constant for the trial period of 10 hours ($r^2 = 0.9919$, n=10). Light intensity (50 – 350 µE s^{-1} m^{-2}) and spectra have a significant effect on survival and length and weight increase.

With fluorescent light the survival rate went down to 70%, whereas with HQL and HQI lights, survival rates were 90% and 100%, respectively. The condition factor, relating to the overall growth condition of the clams (wet weight/shell length), developed best under HQI light conditions. Fastest length and weight increase of *T. maxima* was achieved with HQI lights (Tab. 1).

Table 1: Growth of T. maxima (n=10) with different light sources, FL= fluorescent lamp.

	HQI	HQL	FL
Length (mm day^{-1})	0.0188 ±0.007	0.007 ± 0.005	0 ±0.003
Weight (g day^{-1})	0.08 ±0.04	0.01 ±0.02	0 ±0.02
Survival rate (%)	100	90	70

Nutrient concentrations only play a role when clams are kept in optimum light conditions. In this case fertilization with NH_4^+ and PO_4^{3-} is more effective for zooxanthellae development or growth than feeding with food algae. However, the concentration of zooxanthellae is only moderately and not significantly increasing with increasing nutrient concentration (from 6 x10^8 g^{-1} wet weight at 0 µM NH_4^+ to 9.5 x10^8 g^{-1} wet weight at 100 µM NH_4^+ under HQI light).

The six HQI light sources provided the same spectrum but differed slightly in intensity (Fig. 4). Resulting ETR rates of different clams were almost identical and display a saturation level of 250 µE $m^{-2}s^{-1}$ at PAR levels of 2500 µq $m^{-2}s^{-1}$ (Fig. 5).

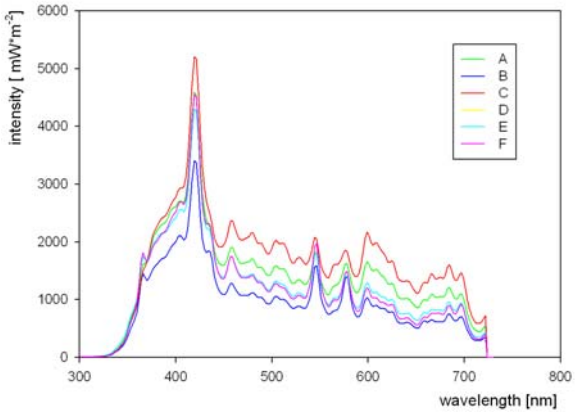

Figure 4: Spectrum comparison of HQI light in six different aquaria.

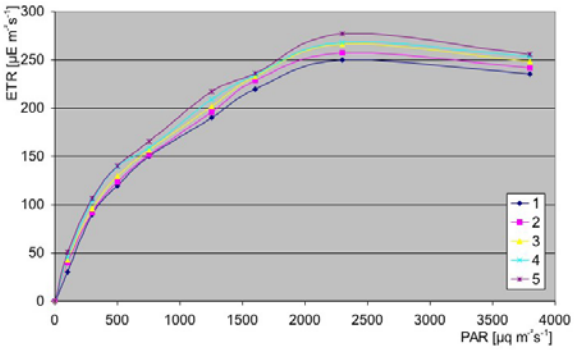

Figure 5: ETR of five different giant clams (*T. maxima*) as a function of PAR.

The oxygen consumption rates (OC) of the same individuals increased slightly over five months, from 0.011 ml O_2 $g^{-1}h^{-1}$ to 0.017 ml O_2 $g^{-1}h^{-1}$. Smaller sized specimens had a higher OC (0.024 ml O_2 $g^{-1}h^{-1}$ to 0.027 ml O_2 $g^{-1}h^{-1}$, Fig. 6).

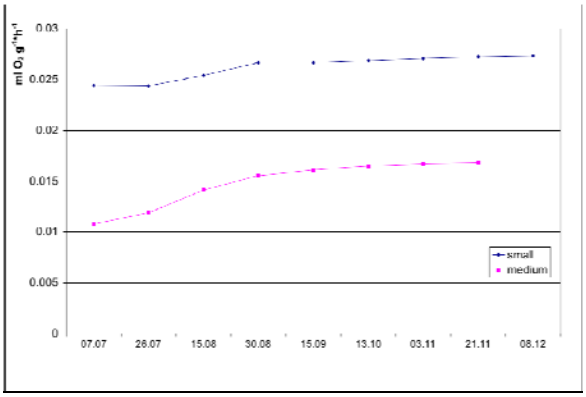

Figure 6: Oxygen consumption rates of the same individuals of *T. maxima* over five months.

Discussion

In this study clam survival is mainly dependent on the lighting conditions; nutrients play a secondary role, at least under experimental conditions and at that age. Only when the clam is kept under optimum conditions clam growth responds positively to the nutrient enrichment. This is in line with other studies (Griffiths and Streamer 1988; Klumpp et al. 1992; Klumpp and Griffiths 1994; Klumpp and Lucas 1994), which have shown that high light intensities may be able to satisfy the basic nutritional needs of clams. But according to Fitt et al. (1993) this strong reliance to zooxanthellae as a source of carbon makes light a limiting factor to growth and survival.

The positive response to nutrients also applies to its symbionts, shown in this study by increased zooxanthellae number, also detected by Braley et al. (1992). The fact that the concentration of zooxanthellae is only moderately and not significantly increasing with increasing nutrient concentration could be due to the short experimental period of 21 days. Ambariyanto and Hoegh-Guldberg (1997) detected a significant effect on zooxanthellae only after three months of nutrient enrichment. The uptake mechanism of zooxanthellae in closed systems might be a problem. The results also show that the symbionts do not have direct access to the nutrients from the water, as proposed by Belda and Yellowlees (1995).

It is not clear yet, why the nutrient uptake, which was also tested under unfavourable light conditions by Hernandez (2006), seems to remain constant, although it is not translated into growth, better survival rates or increase in number of zooxanthellae. Nutrients seem to play a role in the synthesis of the shell, as Schlüter (2005) found changes in shell stability. Marubini and Atkinson (1999) suggest that inorganic nutrients may slow down the calcification.

Similar observations on little effect of nutrient concentrations on clam growth were also made by Ambariyanto and Hoegh-Guldberg (1997) and Sparsis et al. (2001), which is in contrast to results from Hastie et al. (1992), Braley et al. (1992) and Fitt et al. (1993), where a significant contribution of nutrient enrichment to the biomass of the clams was seen. This might be due to the fact that the authors had maintained a continuous flow of nutrients into the water for more than 3 months, while in this study and the above-mentioned studies by Ambariyanto and Hoegh-Guldberg (1997) and Sparsis et al. (2001), spiking only lasted up to 2 to 3 hours a day and this only for a short period of two months. To get more details, additional investigations also on chlorophyll concentrations are planned.

This study has shown that effluents from aquaculture tanks can be supplied to these animals as a source of nutrients, offering possibilities for polyculture. The fact that clams tolerate high concentrations of NO_3^- (pers. observation) would even allow for joint culture with ornamental fish. However, the use of clams for removal of large quantities of nutrients has limits and needs to be investigated with larger specimens and different species. Due to the fact that effluents are always a mixture of several nutrients, the most suitable ratios of N:P need to be tested and adjusted accordingly.

From previous investigations (Lucas 1994) it is known that several specimen being kept in one aquaria, are able to deplete nutrient values considerably. This also applies to calcium, as both calcium and phosphate are needed for the shell production. Growth is slowed down considerably, when either Ca^{2+} or PO_4^{3-} is not available (Schlüter 2005). This needs to be considered when relying entirely on effluents for nutrient supply; aquaculture effluents do not necessarily contain Ca^{2+} in sufficient concentrations.

It is **concluded** that: clams need optimal light conditions, significant nutrient removal is possible but limited, OC rates are size dependent. In future experiments also adult specimens need to be tested.

The aquaculture of giant clams should be increased, both for ornamental and for bio-cleaning purposes. Because of dwindling populations of natural stocks, future work of ZMT will concentrate on improving aquaculture conditions and exploring physiological performance of giant clams under varying environmental conditions, including stress situations and climate change scenarios. Some experiments conducted only with *T. maxima* and *T. derasa* need to be repeated with *T. gigas*, because of its exceptional fast growth.

Future experiments will concentrate on different size ranges and on the reaction to physical, chemical and biological stress, also by modelling effects induced by climate change.

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Secondary succession of coral reef communities at Urasoko Bay, Ishigaki Island, the Ryukyus (southern Japan)

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Abstract: Observations of secondary succession of coral reef communities have been carried out at Urasoko Bay, Ishigaki Island, the Ryukyus, southern Japan from 1995 to 2007. Three stations have been permanently established on the outer reef flat, the inner flat, and inshore moat, with four permanent quadrats at each station. At the start of the study in September 1995, all organisms were scraped from the substrate surface. Thereafter, changes in percent cover of hermatypic corals and marine algae have been quantified annually. During the 12-years period, three bleaching events and a typhoon occurred, causing significant disturbance to these sites. Our long-term monitoring results show that recovery to a coral-dominated community (mainly *Acropora* spp.) requires 4-6 years and that transient domination by crustose coralline algae for 2-3 years facilitated coral recovery. It is suggested that crustose coralline algae play a role in construction of firm substrata for settlement and growth of hermatypic corals. The inshore station, which is dominated by rubble, remains nearly in a permanent state of disequilibrium between corals and algae.

Key words: Algae, Coral reef communities, Crustose coralline algae, Ecological succession, Ryukyu Islands.

Introduction

Recently, the health of coral reefs throughout the world has deteriorated due to various natural and human stresses (Hughes 1994; Wilkinson 1998, 2000, 2002; Bellwood et al. 2004). Coral reefs in the Ryukyus have also been gradually destroyed by pollution, excess sedimentation, bleaching and predation by *Acanthaster planci* (L) as well as by other factors (Nishihira 1987; Fujioka 1999; Yamazato 1999; Ohba et al. 2004, 2006). Restoration of coral reefs is a costly and uncertain endeavor, the techniques of which remain largely experimental (Omori and Fujiwara 2004; Precht 2006; Omori et al. 2008). Results are typically evaluated on an empirical basis with only a marginal understanding of the underlying causes facilitating or inhibiting recovery to a coral-dominated state. Long-term monitoring studies of reefs provide a temporal window on changes in community composition, including natural responses to disturbance. However, such long-term, place-based studies are relatively uncommon. At Urasoko Bay, we have followed secondary succession for 12 years (Fujioka 2002; Fujioka et al. 2006), which includes three bleaching events and a typhoon. Here we summarize the most important findings from this ongoing study with respect to natural resilience and the coral-dominated state in the face of these events.

Materials and Methods

Secondary succession of coral reef communities was monitored at three stations established in Urasoko Bay at Ishigaki Island in the Ryukyu Archipelago. These three stations represented the (A) outer reef flat, (B) inner reef flat, and (C) inshore-side moat (Fig. 1-2). The depth of each station was 0.5-1.5 m and the survey was carried out by SCUBA diving and snorkeling. Four permanent quadrats (1 m x 1 m) were put in place at each station. Subsequently, all organisms were scraped from the substrate surface in all quadrats in September 1995 and again in March 1996. The percent cover, number of species and of colonies of hermatypic corals in each quadrat were measured around in September each year from 1994 to 2007. The percent cover of marine algae in each quadrat was also measured in March 2002 and around September from 2002 to 2005. Percent algal cover from 1994 to 2001 and 2006 to 2007 was evaluated from underwater photographs taken at each quadrat. Percent cover is reported as the average of the four quadrats at each station.

Results and Discussion

In 1994, before the reef surfaces were scraped, tabular *Acropora* dominated all permanent quadrats at Station A with a secondary mix of corymbose and branching *Acropora*. Little algae or other invertebrates were observed (Fig. 3). Various growth forms of *Acropora* formed a mixed assemblage in two of the permanent quadrats at Station B. The rubble derived from branching corals filled the other two quadrats at Station B, and also two quadrats at Station C. Small filamentous, creeping and encrusting algae were observed growing on the rubble. Massive *Porites* dominated the two other quadrats at Station C, as well as a mixture of *Acropora, Pocillopora,* Faviidae and other species.

The percent cover of hermatypic corals at Stations A, B and C increased and reached 43%, 28%, and 24%, respectively, in 1998, three years after the substrate surfaces were scrapped. However, a large-scale coral bleaching event took place in coral reefs worldwide in 1998 (Wilkinson 1998, 2000), which also affected the Ryukyus reefs (Fujioka 1999;

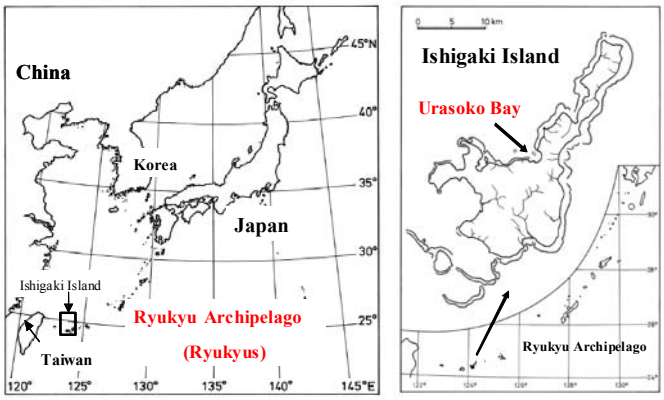


Figure 1: Maps of East Asia and Ishigaki Island in the Ryukyus (southern Japan) showing the study area of Urasoko Bay.

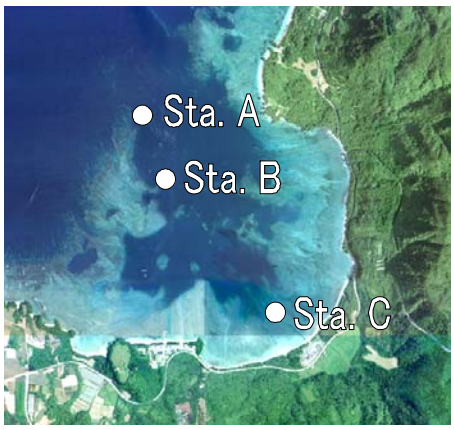


Figure 2: Aerial photograph of the three stations within Urasoko Bay.

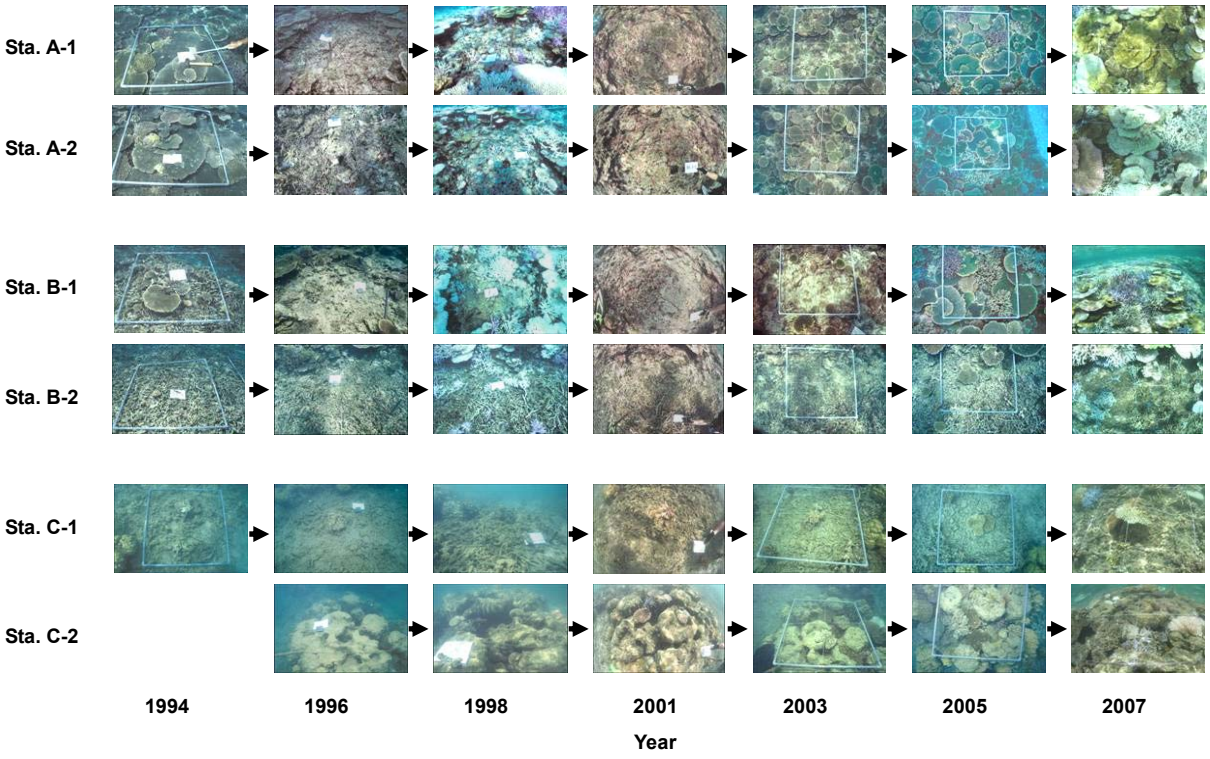


Figure 3: Underwater photographs showing changes in the reef communities in two of the four quadrats at each station in Urasoko Bay. The substrate surfaces in each quadrat were scraped in 1995. A photograph of the Station C-2 in 1994 was not taken.

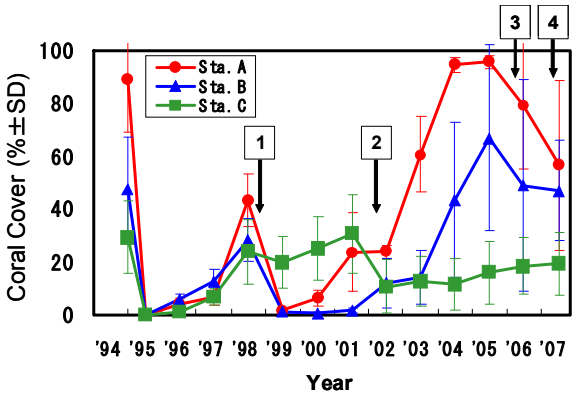


Figure 4: Changes in the percent cover of hermatypic corals at each station in Urasoko Bay from 1994 to 2007. The substrate surfaces in each quadrat were scraped in 1995. Arrows indicate disturbance events: 1. Outbreak of large-scale coral bleaching; 2. Typhoon; 3 and 4. Coral bleaching.

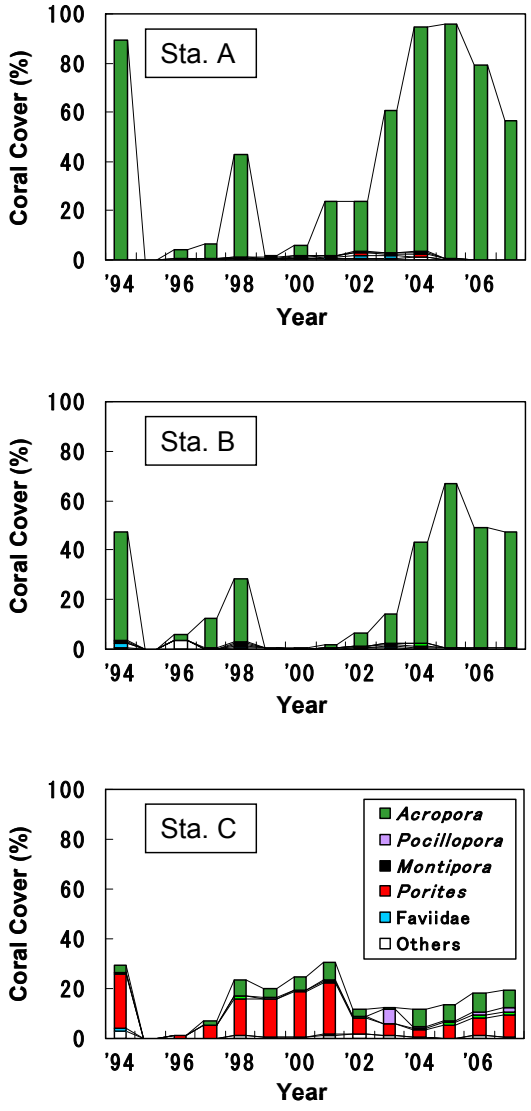


Figure 5: Changes in the percent cover of coral taxa at each station in Urasoko Bay from 1994 to 2007.

Yamazato 1999). During this bleaching event, most of hermatypic corals at Stations A and B died between summer and fall of 1998, although the *Porites* colonies survived at Station C (Fig. 3-4). By 1999, the percent cover of hermatypic corals at Stations A and B was reduced to 1.6% and 0.8%, respectively.

The percent cover of hermatypic corals at Stations A, B, and C increased gradually after the coral bleaching event in 1998 until 2002, when the cover stopped increasing or was again reduced by the damage caused by a large typhoon (No. 16 in 2002). The percent cover of hermatypic corals at Station A rapidly started increasing in 2002 and reached 95% in 2004. The cover at Station B also rapidly increased and reached 67% on average in 2005, with a maximum of 99.5% observed in a quadrat located on the rocks (Fig. 3, Station B-1). The cover at Station C reached a maximum of 31% in 2001, then decreased to only 11% in 2002, followed by a gradual increase to 19% in 2007. Coral bleaching occurred again in the southern Ryukyus in 2006 and in 2007, causing the percent cover of hermatypic

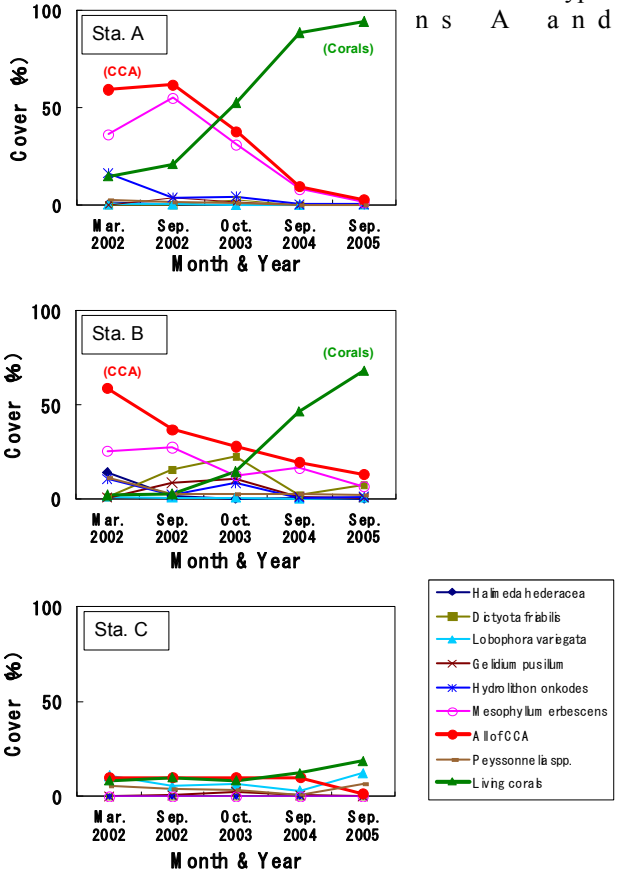


Figure 6: Changes in the percent cover of algae and total corals (- ▲ -) at each station from March 2002 to September 2005. CCA: Crustose coralline algae.

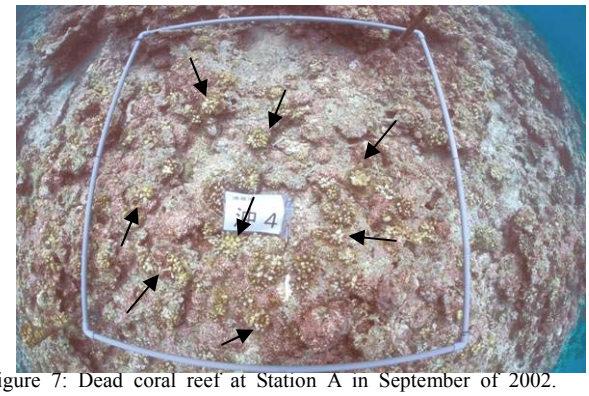


Figure 7: Dead coral reef at Station A in September of 2002. Crustose coralline algae (pink) were luxuriantly growing on dead corals and many small recruits of corals (pale brown: arrows) were beginning to be settled.

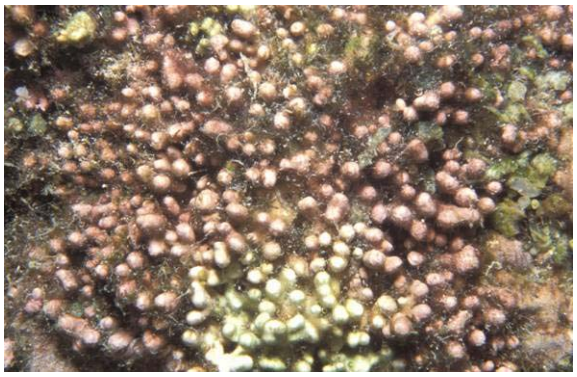
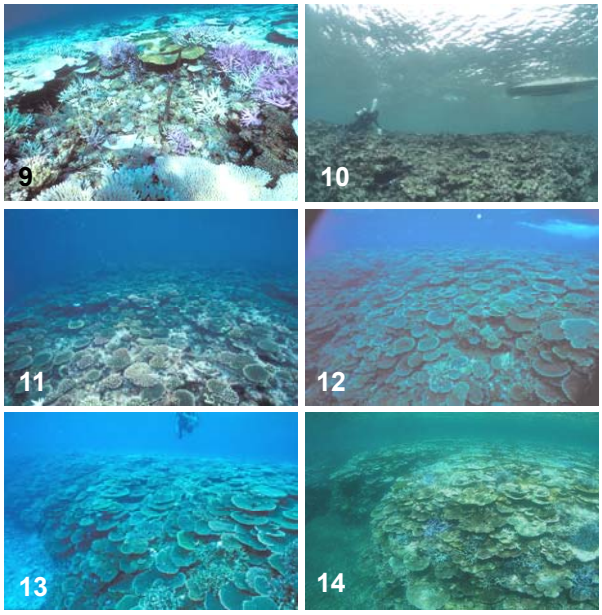


Figure 8: A crustose coralline alga, *Mesophyllum erubescens*, and filamentous algae growing on dead corals at Station A in September of 2002.

B to decrease to 57% and 47%, respectively, in 2007 (Fig. 4).

The changes in species composition at each station are shown in Fig. 5-6. The sequence of secondary succession was quite different at Station C compared to Stations A and B (Fig. 5). Coral recruits, as well as small filamentous, creeping, encrusting algae and crustose coralline algae immigrated and began to grow at Stations A and B in 1996, a year after the substrate surface had been scrapped. This recovery was highlighted by the resettlement of *Acropora* recruits. *Acropora* basal discoid parts were observed from a half to one year after the settlement. These recruits began to grow into an upright morphology from the center of their basal sections in 1997 and 2001. The growth rate of the upright part of *Acropora* was rapid, with an increase in the diameter of the colonies of 30-50 cm by 2005. When the secondary succession on the rocks at Stations A and B reached a climax (i.e., coral domination), the species composition and cover of the hermatypic coral community were similar to those of the original community in 1994. At Station C, *Acropora* recruits were much fewer than those in Stations A and B,



Figures 9-14: Underwater photographs showing an alternation of damaged and recovered coral reefs at Station A in Urasoko Bay from 1998 to 2007. 9. Large-scale coral bleaching in September of 1998. 10. Dead coral community in September of 2001. 11. Recovered coral community in October of 2003. 12. Almost recovered coral community in September of 2004. 13. Completely recovered coral community in September of 2005. 14. Coral bleaching occurred again in August of 2007.

whereas the recruits of many kinds of *Porites, Pocillopora, Montipora,* Faviidae and other genera were present on the rocks (Fig. 5). In Particular, massive *Porites* dominated from 1997 to 2002. The species composition and cover of hermatypic corals at Station C in 2001 were similar to those of the original community in 1994.

Green algae such as *Halimeda hederacea*, brown algae such as *Dictyota friabilis* and *Lobophora variegata*, and red algae such as *Gelidium pusillum, Hydrolithon onkodes, Mesophyllum erubescens* and crustose coralline algae (CCA) temporarily appeared and grew luxuriantly during 1995 to 1997 and during 1999 to 2003, before the hermatypic coral community became dominant at Stations A and B (Fig. 6-8). Crustose coralline algae kept growing on the substrate at all stations. In particular, a thick crustose coralline alga, *Mesophyllum erubescens* (Fig. 8), had dominated on the rocks at Stations A and B from 1999 to 2002. The percent cover of crustose coralline algae gradually decreased with the growth of corals (Fig. 6). Previous research highlighted the importance of crustose coralline

algae as coral settlement inducers (Morse et al. 1996). In addition, coralline algae may construct the firm substrates for settlement and growth of hermatypic corals by covering the surface like a cement, and therefore may possibly inhibit the perforation of many micro-organisms which damage the framework of substrates. Similarly, it is supposed that, in the Ryukyus, crustose coralline algae may play an important role in facilitating the settlement of coral recruits and acting as an important member of the reef-builder functional group.

On the rocks around the outer reef flat in Urasoko Bay, two sequences of secondary succession are recognized. In the first case, the hermatypic coral community recovered directly between 1995 and 1998. In the second case, the coral community recovered after a phase of algal overgrowth that lasted for 2-3 years between 1998 and 2005. The climax of the secondary succession, where *Acropora* community is formed, seems to be reached 4-6 years after the start of the succession, as long as the coral reefs remain in good health and are not subjected to large disturbance such as coral bleaching, sedimentation, typhoons or other bad factors (Fig. 9-14). In contrast, the coral community found on the rubble and rocks located at the inshore-side of Urasoko Bay seems to take longer to reach the climax of the secondary succession. It is considered that the recruitment of coral planulae may be reduced in the moat, and that the loose rubble is an unstable substrate which limits coral planulae settlement. The sequence and speed of the secondary succession were influenced by substrate stability and the various sources of disturbance that characterized each habitat.

In conclusion, long-term monitoring studies provide insights into the cause of successional differences in response to disturbances that delay or enhance coral domination as an alternate stable state. For fast growing corals such as *Acropora*, recovery from catastrophic events may be ultimately impossible, but as shown here, even with periodic bleaching events and storms, recovery is possible in a timeframe of a few years. Given that coral bleaching and storms are ongoing processes, it remains paramount that anthropogenic impacts such as pollution and dredging be curtailed.

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Scleractinian coral recruitment to reefs physically damaged by ship groundings

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Abstract. The southeast Florida reef system faces a number of stress factors, among which ship groundings are one of the most physically damaging. Portions of the Florida reef tract located near Port Everglades, Broward County, Florida, USA have been damaged by ship groundings. In 2004, physical damage of more than 30,000 m^2 was caused by the groundings of two large cargo ships, the MV *Eastwind* and MV *Federal Pescadores.* The present study was designed to measure differences of scleractinian coral recruitment patterns (recruit diversity and richness) and rates to these injured sites in comparison to undamaged reef sites. Coral recruitment rates were measured on unglazed ceramic tiles deployed for a period of one year from February 2007 to February 2008 at five different locations: three control sites (including a high coral cover site), and the two ship grounding sites. Morphology and genetic markers including CO1 and cytb were used to identify the coral recruits. A whole genome amplification kit (REPLI-g, Qiagen) was used to obtain sufficient amounts of DNA. Results revealed low recruitment rates (0.5-2.7 recruits m^{-2} yr^{-1}) to the studied sites, suggesting a low potential for recovery of the damaged areas.

Key words: reef groundings, coral recruitment, genetic markers

Introduction

Coral reefs around the world have been affected by anthropogenic changes in the environment, resulting in a general pattern of decline worldwide. A substantial number of these stressors also affect the Florida coral reef system, and ship groundings have been found to be particularly destructive (Jaap 2000; Collier et al. 2008). Reef areas located near Port Everglades, Broward County, FL have been severely impacted due to the location of a portion of the port anchorage area between two major reef tracts: the middle and outer reefs. Navigational error and inclement weather have resulted in a number of ship groundings and anchor damage events. From 1993 to 2006, there were 11 groundings and 6 anchor drag cases, resulting in more than 40,000 m^2 of coral reef injury in the vicinity of Port Everglades (Collier et al 2007, Banks et al. 2008). In 2004 the most severe impacts were groundings of two cargo ships: the MV *Eastwind* in March and the MV *Federal Pescadores* in October, which destroyed more than 30,000 m^2 of reef (Melendez 2004; Flesher 2004).

Coral recruitment is a vital step in the natural recovery process of injured reefs. Thus, understanding the process of coral recruitment to these areas is essential in evaluating the ability of reefs to recover (Smith 1992; Tougas and Porter 2002; Glassom et al. 2004). At 26°N latitude, Broward County is near the northern range limit for many scleractinian coral species, which may lead to an assumption of low recruitment rates and thus slow recovery. The present study was designed to estimate scleractinian coral recruitment rates and patterns (species diversity and richness) to damaged reef sites three years after the initial impacts of the two cargo ships MV *Eastwind* and MV *Federal Pescadores*. It also aimed to determine differences in coral recruitment patterns and/or rates between undamaged parts of the reef and sites which were destroyed by ship groundings. This study has provided the first estimation of scleractinian coral recruitment rates on reef sites off the coast of Broward County, FL.

One of the challenges of coral recruitment studies is properly identifying coral recruits. Previous researchers have typically been able to morphologically categorize recruits only to the family level because of the small size (< 1 to several mm) of many recruits (Baggett and Bright 1985; Harriott 1992; Tougas and Porter 2002). However, genetic markers have been used to overcome the difficulty in morphological identification of early life stages (gametes, larvae, juveniles) of a number of marine

organisms (Neigel et al. 2007). The cytochrome *c* oxidase subunit 1 (CO1) mitochondrial gene has been used to identify coral recruits settled on ceramic tiles (Shearer and Coffroth 2006) and revealed a great advantage in the ability to identify recruits as small as 1 to 2 mm in diameter. In this study, genetic markers including CO1 and cytochrome b (cytb) genes were used to supplement the morphological identification of coral recruits to a lower taxonomic level.

Material and Methods

Coral recruitment rates were measured on unglazed ceramic tiles deployed off the coast of Broward County for a period of one year from February 2007 to February 2008. A total of 480 ceramic plates (8 per array, Fig. 1) were deployed, resulting in 22.3 m^2 of settling area under study.

Figure 1. Settlement plate array design. Twelve arrays deployed to each of 5 sites for a period of one year.

The settlement plate arrays were deployed at five sites (12 arrays per site): three control sites (including one high coral cover site) and two ship grounding sites (*Eastwind* and *Federal Pescadores*) (Table 1). After 12 months on the reef, the plates were collected, transported in coolers and frozen (-20° C) until microscopic and genetic examination. The tiles were examined for coral recruits under a dissecting microscope at 12X magnification, and the recruits found were identified by morphological characteristics to family or genus level according to the identification keys in Budd et al. (2006) and Smith (1948).

Each scleractinian spat was then scraped with a razor blade and preserved in a saturated sodium chloride 2.5M EDTA, 20% dimethyl sulfoxide (DMSO) buffer for genetic analysis. DNA extraction was completed with a DNeasy isolation kit (Qiagen). For samples with low concentration of extracted DNA, a whole genomic DNA was amplified with REPLI-g kit (Qiagen). The CO1 was amplified by the polymerase chain reaction (PCR) using coral–specific primers and cycling conditions suggested by Fukami et al. (2004).

The cytb and ITS genes were amplified using newly designed primers; CYTBF: 5'-GGGTGTTT TTTGTCBATGCATTAT-3', CYTBR: 5'CCCAATT TATTTGGTATCGAACGCA-3', ITSF:5'GGGGAC AGAGMGTCGGAT-3', ITSR:5'-TCCGGGKAGAA AGTGCTTCT-3'.

Table 1. Location of five reef sites in Broward County, FL; CS1 = control site one, CS2 = control site two, HC1 = high coral cover site, EW = *Eastwind* and FP = *Federal Pescadores*

SITE	LATITUDE	LONGITUDE	DEPTH (m)	REEF TYPE
CS1	26°09.625'N	80°05.306'W	6.5-7.5	INNER
CS2	26°10.073'N	80°05.265'W	6.5-7.5	INNER
HC1	26°08.857'N	80°05.763'W	6.5-7.5	RIDGE COMPLEX
EW	26°07.042'N	80°05.549'W	9.0-10.0	INNER
FP	26°06.747'N	80°05.504'W	11.5-12.5	INNER

The PCR protocol for the above primers was 35 cycles at 94°C for 45s, 50°C for 45s, and 72°C for 90s. Following the work of Shearer and Coffroth (2004), the restriction fragment length polymorphism (RFLP) patterns of the CO1 gene were used for partial identification of the recruits. First, virtual RFLP patterns for major scleractinian coral species were created with a Web-based nucleic acid analysis tool (http://workbench.sdsc.edu/) using CO1 sequences available in GenBank. The CO1 RFLP patterns of recruits were created by Taq1 restriction digestion of the amplified gene and then compared to the virtual patterns of known species. Because of the low interspecific variation in CO1 gene sequences among scleractinian corals (especially in the family Faviidae), the CO1 RFLP was useful to differentiate only two of the coral species from the genus *Porites*. CO1 and cytb genes acquired from coral recruits that were not distinguished by RFLP patterns were sequenced and blasted to find the best possible match in GenBank. In case of low concentration or low purity of the PCR results, products were cloned with TOPO TA Cloning kit (Invitrogen). The final identification was made based on the combined morphological analysis RFLP patterns and BLASTN queries (www.ncbi.nlm.nih.gov/BLASTN).

Results

A total of 33 coral spat were found on 478 tiles (two tiles were lost). The overall recruitment rate was estimated to be 1.5 recruits m^{-2} yr^{-1}, ranging from 0.5 recruits m^{-2} yr^{-1} at the *Federal Pescadores* ship grounding site to 2.7 recruits m^{-2} yr^{-1} at both the control site 1 and high coral cover site (Fig. 2). A one-way analysis of variance showed no significant difference among recruitment rates from the five studied sites, likely due to a small sample size. The lowest number of recruits settled on tiles located at the *Federal Pescadores* ship grounding site (2 recruits) and the highest number of recruits were found on tiles from control site 1 (11 recruits) and the high coral cover site (10 recruits) (Table 2).

The identification process of scleractinian coral recruits using genetic markers was successful for all but one sample. The small size of the recruits, which in several cases were less than 1 mm in diameter, resulted in extraction of very small amounts of DNA. Another challenge was related to recruit DNA contamination with zooxanthellae DNA. The easiest markers to be obtained from this poor quality DNA were CO1 and cytb mitochondrial genes.

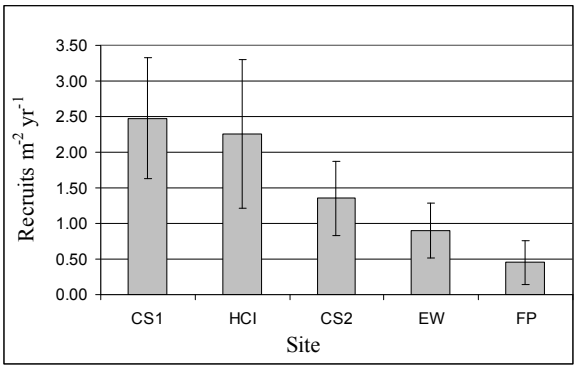

Figure 2. Scleractinian coral recruitment rates found on settlement tiles located on five reef sites in Broward County, FL. Mean values are calculated per array (n=12) with error bars indicating standard errors. CS1 = control site one, CS2 = control site two, HC1 = high coral cover site, EW = *Eastwind* and FP = *Federal Pescadores*.

Amplification of nuclear genes such as ITS turned out to be more challenging and was only successful for a few of the largest recruits. One method of improving results was to amplify the whole genome using a REPLI-g kit from Qiagen which allowed an increase in the concentration of DNA template (Table 3). This technique showed potential to be a useful tool when very small samples such as gametes, larvae and other early life stages are involved, since target genes could still be amplified.

Table 2. Comparison of scleractinian coral recruitment rates and patterns among five reef sites. CS1 = control site 1, CS2 = control site 2, HC1 = high coral cover site, EW = Eastwind grounding site, and FP = Federal Pescadores grounding site.

SITE	NUMBER OF RECRUITS	NUMBER OF GENERA	NUMBER OF Porites spp.	SHANNON WEINER DIVERSITY INDEX	TOTAL RECRUITS m^{-2} yr^{-1}
CS1	11	3	10	0.92	2.96
HCI	10	3	7	1.10	2.69
CS2	6	4	4	1.36	1.61
EW	4	1	4	0.00	1.08
FP	2	1	2	0.00	0.54

The restriction digestion performed on the CO1 gene (total 742 bp) with Taq1 restriction enzyme resulted in 4 different RFLP patterns (Fig, 3). Patterns designated with letters B (2 fragments: 446, 296 bp) and C (3 fragments: 330, 298, 116 bp) were species-specific for *Porites astreoides* and *Porites porites*, respectively. Patterns A (2 fragments: 613, 129 bp) and D (3 fragments: 374, 296, 72 bp) were characteristic of multiple species, so recruits with these two patterns were chosen for better resolution via CO1 and cytb sequencing analysis.

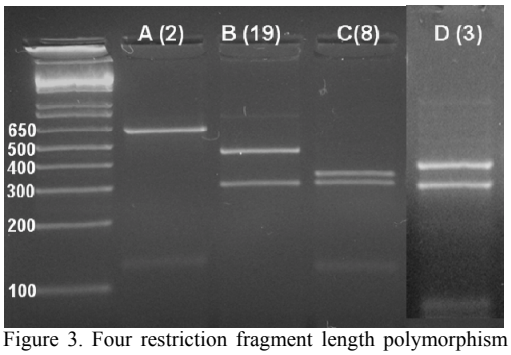

Figure 3. Four restriction fragment length polymorphism patterns named A, B, C, and D created from the CO1 gene amplified from scleractinian coral recruit DNA samples and digested with Taq1 restriction enzyme. The number in parenthesis indicates the number of times the pattern was found.

Table 3. DNA concentrations extracted from coral recruits, and DNA concentrations of the same samples after whole genome amplification determined by Nanodrop ™

SAMPLE	DNA (ng*µL^{-1})	DNA AFTER REPLI-g (ng*µL^{-1})
CS154	0.42	341.85
HCI52	1.36	321.90
CS144	1.69	218.00
CS212	3.59	250.94
CS146	3.86	333.86
CS261	6.50	246.38
HC126	10.17	270.72

There were a total of four coral recruit genera found on the tiles. The most dominant coral species recruiting to all five sites were *Porites astreoides* and *Porites porites* comprising 82% of all the recruits. Twelve percent of the coral recruits consisted of species from the genus *Agaricia,* and the remaining 6% (two recruits) were *Montastraea cavernosa* and *Colpophyllia natans*. The last two mentioned were the only two recruits of reef framework-building corals. These recruits were found on tiles from the high coral cover site which had the highest diversity (all four genera represented) of all the studied sites (Table 2).

Discussion

Overall coral recruitment rates and patterns

The present study on reef sites off Broward County, FL demonstrated extremely low recruitment rates, between 0.5 to 2.7 recruits m^{-2}. The low recruitment rates to these reefs were not surprising due to their location on the northern extent of the Florida reef tract which is characterized by relatively low coral cover.

In Florida and the Caribbean coral recruitment rates have been declining most likely as a consequence of the overall decline of coral population densities (Porter et al. 1988; Jaap et al. 2000; Santavy et al. 2005, Hughes and Tanner, 2000). Through meta-analysis, Gardner et al. (2003) have estimated an 80% reduction in coral cover in three decades between 1977 and the present for the entire Caribbean basin. Extensive coral cover losses certainly have led to a decrease in coral breeding stocks, thus resulting in reduced larval supply and low recruitment rates.

The most dominant coral species recruiting to the studied reef sites were *Porites astreoides* and *Porites porites* which constituted 82% of all the recruits. The next dominant species were from the genus *Agaricia* (12%). The trend of dominant recruitment of agariciid and poritiid corals has been documented in several other studies of coral recruitment on Florida and Caribbean reefs (Bak and Engel 1979; Rogers et al. 1984; Smith 1997; Hughes and Tanner 2000; Tougas and Porter 2002; Shearer and Coffroth 2006). The recruitment patterns found in previous studies have been explained by either: a) the existence of two different life histories exhibited by scleractinian corals (broadcasting vs. brooding) (Bak and Engel 1979) or b) the Allee Effect of low population densities (Knowlton 2001).

The first theory states that coral species which are massive, long-lived, reef frame-builders and usually broadcast spawn their gametes into the water have low recruitment rates and are represented by a low number of juvenile colonies. The low recruitment rate, however, is then compensated by lower post-settlement mortality and good competing and surviving abilities (Bak and Engel 1979; Smith 1992 1997; Hughes and Tanner 2000). In contrast, small-sized, short-lived species that brood their larvae exhibit higher recruitment rates but also suffer from higher post-settlement mortality rates. The adult community structure with the dominance of massive broadcast spawning species like *Montastraea* spp. and *Diploria* spp. over small, brooding species such as *Porites* spp, *Agaricia* spp. and *Favia* spp. is thus the result of the two opposite life strategies.

The Allee Effect mostly affects broadcasting species which generally do not self-fertilize, so high gamete densities are necessary for successful fertilization and reproduction (Knowlton 2001). Low population densities of these species lead to asynchronous spawning events (limited hormonal regulation), low gamete densities and consequently to the increase in reproductive failure. Brooding species can self-fertilize, and their larvae can settle within hours to days of release. Therefore, they are more likely to persist through periods of low densities. The Allee Effect creates a major shift in the structure of coral adult communities from those dominated by framework builders toward those dominated by non-framework opportunists such as *Porites*. Both the differing life history strategies and the Allee Effect could be the cause of *Porites* dominated recruitment patterns in Broward County, FL.

Recovery process in terms of recruitment rates on ship grounding sites

Ship groundings on coral reefs cause not only serious damage to the reef builders, but also result in a loss of habitat for other animals, consequently leading to barren areas. The problem, however, may be further intensified by other stress factors (climate change, disease, pollution, eutrophication and sedimentation) which decrease coral population size, impact the size of coral breeding stocks, larval supply and recruitment rates, and increase post-settlement mortality rates, consequently resulting in unlikely recovery of the damaged sites.

The present coral recruitment data showed that a small number of coral recruits settle on tiles located on the studied ship grounding sites suggesting a very slow recovery process and unlikely recovery success. Additional studies of coral recruitment involving artificial and natural substrates are required to confirm the low recovery potential of these and other physically damaged sites in Broward County, FL.

Genetic identification

This study demonstrated that genetic markers help overcome the difficulty in morphological identification of early life stages such as small juvenile coral recruits. We confirmed the usefulness of the CO1 gene in partial species or family level identification of scleractinian coral recruits as it was developed by Shearer and Coffroth (2006). RFLP analyses of the CO1 gene allowed differentiation between at least two species in the coral genus *Porites*. The low nucleotide polymorphism in CO1 was, however, insufficient to distinguish some closely related coral species (Shearer and Coffroth 2007; Neigel 2007), but a better genetic marker has not yet been developed for this purpose.

This study tested other genetic markers including cytochrome b and internal transcribed spacer region (ITS) for species identification of corals in the early developmental stage. Coral-specific primers were designed and tested in order to avoid amplification of zooxanthellae genes. Nuclear markers were difficult to amplify from the small amounts of DNA. Similar to the CO1 gene, the cytb gene showed limited genetic variation, which limits the number of species specific RFLP patterns. However, the combination of obtaining both gene sequences allowed better species

identification, which could be improved with more markers.

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Underestimated Eroder among Reef Fishes – Experimental Comparison between *Ctenochaetus striatus* and *Acanthurus nigrofuscus* (Acanthuridae)

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Abstract. The acanthurid fish species *Ctenochaetus striatus* and *Acanthurus nigrofuscus* are common detritivore browsers. Less is known about their erosion potential. We offered coral limestone for grazing in aquarium experiments and recorded the feeding behaviour by video. *C. striatus* produced 15 times more calcareous sediments compared to *A. nigrofuscus* despite of a 5 times higher biting rate of *A. nigrofuscus*. Whereas *A. nigrofuscus* performed careful picking bites only, *C. striatus* showed soft sweeping of the surface alternating with chafing the substrate with grasping bites. Hereby a special palate structure (bow of hard knobs in the upper jaw) is engaged which allows to rasp the reef substrate. The measured eroded masses per bite and the number of bites per individual were related to the population densities of the two species on three reef crests in the Northern Red Sea. Based on these extrapolations, *A. nigrofuscus* attains maximum erosion rates of 2.6 g m^{-2} yr^{-1} and may therefore not be regarded as a relevant bioeroder, but *C. striatus* achieves erosion rates of up to 70.0 g m^{-2} yr^{-1} which is in the same order as that of the co-occuring sea urchin *Diadema setosum.*

Key words: Detritivore acanthurids, jaw morphology, bioerosion, Red Sea

Introduction

Coral limestone plates which were set up for colonization experiments at Aqaba (Jordan, Northern Red Sea) were found to be eroded after two years by up to 6.12 mm (v.Treeck et al. 1996). The experimental set-up excluded bioeroding sea urchins, but not grazing fish. Surgeonfishes and parrotfishes are the principal grazing groups with many fishes in the latter family capable of scraping and excavating coral limestone (Glynn 1997). In situ video recordings and observations showed that the plates were most frequently grazed by the acanthurid fish *Ctenochaetus striatus* (Quoy and Gaimard 1825) and *Acanthurus nigrofuscus* (Forsskal 1775). Scaridae, however, were rare. Hence, the feeding behaviour of the two species which are known as detritus feeders (Randall and Clements 2001) was investigated in aquarium experiments with supplementary observations in three reefs in the Northern Red Sea.

Trials to run experiments close to the reef in the laboratories of the Ras Mohamed National Park (southern tip of Sinai-Peninsula) could not be sufficiently standardized, since we were not allowed to catch the wanted specimens, but depended on the supply of healthy fishes by Bedouin fishermen. In addition we arranged an experimental set-up in the university aquarium plant in Essen. These data, albeit obtained from a necessarily limited sample size, are presented here and discussed in conjunction with the specific jaw morphology of the two fish species.

Material and Methods

Aquarium trials were run at the University of Duisburg-Essen in spring 2005 with fish obtained from the aquarium trade.Two subadult individuals (8-9 cm TL) of each species were at our disposal. *Favia* plates from the same stock as had been used in the above mentioned experiments at Aqaba were immersed in seawater taken from an aquarium accommodating "living rocks" and invertebrates from the Red Sea, until being covered with a veneer of diatoms, green and bluegreen algae; then they were offered to the individual fish. These had been kept without food for one day to empty the intestinal tract. The browsing activities were recorded by video. After 7-8 hours the plates were removed. The fish, however, remained in the tank for another 12 hours without food. Then all loose particles (i.e. predominantly faeces, not ingested but removed limestone particles, and material generated by handling) were sucked off and collected in 50 µm gauze. These sediments were analyzed for their dry weight carbonate content.

As control, a coral slab of the same size was similarily handled in an aquarium – without fish. To prevent coprophagy during the time when the fish's gastrointestinal tract was emptied a net was installed a few cm above the bottom.

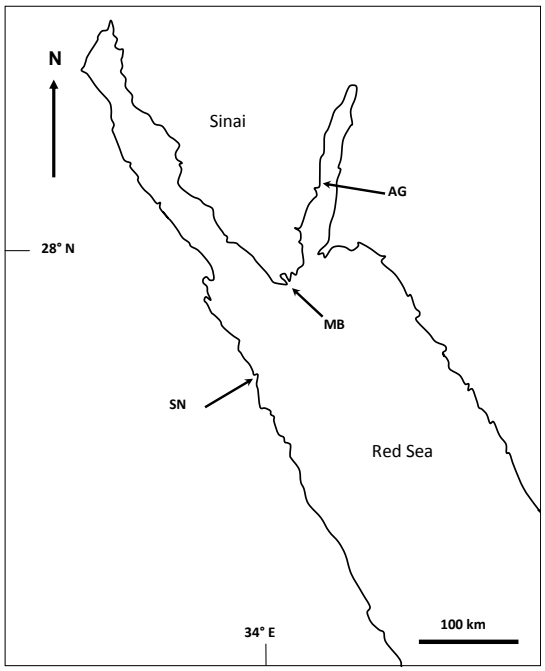

Figure 1: Map of study sites, see text for details.

Field observations were performed by extensive snorkelling on three reefs at different seasons: 1. Abu Galum (AG), 2. Marsa Bareika (MB), and 3. Sharm el Naga (SN, see Fig. 1). Individual fish were observed for several hours at different times of the day counting those bites which clearly occured on hard substrate. Bites on sponges, fleshy algae, and sand covered substrate were ignored. Therefore, the recorded bites per day represent minimum values. The abundance of the acanthurid fish was recorded at the three sites by counting all individuals within 50x5 m transects (3 replicates), placed on the reef crests with a distance of 2.5 m to the edge (English et al. 1997).

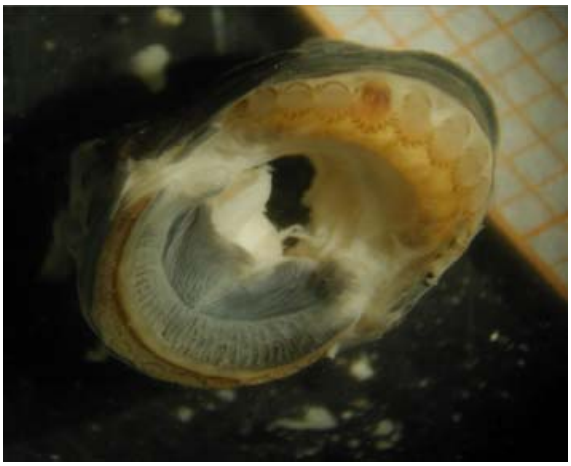
Figure 2: Opened mouth of *A.nigrofuscus*; upper jaw with millimetre grid in background.

Results

Corresponding to the aquarium experiments of Purcell and Bellwood (1993) we found two different feeding techniques.

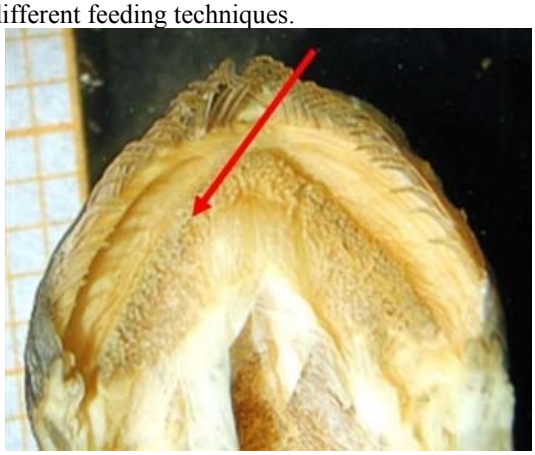


Figure 3: Upper jaw (top) and lower jaw (bottom) of *C. striatus*; arrow points to the knobby structure behind the bristle-like teeth.

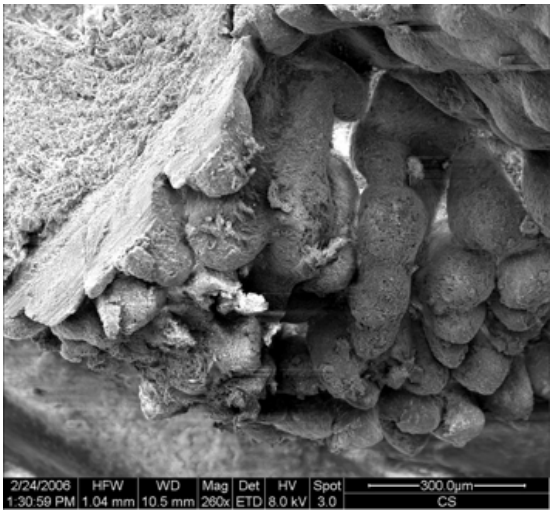

Figure 4: SEM picture of a section of the hard palate structure pointed to in Fig.3.

A.. nigrofuscus grazes with a minimal contact to the substrate. It feeds using quick punctual bites. *C. striatus* feeds opening its mouth up to an angle of 180° sweeping the substrate with its jaw bristles; in addition, forceful grasping bites at which the whole body is shaking and exerting thrust were observed.

Table 1 provides data on number of bites and removed carbonate masses. Although *A. nigrofuscus* showed a five times higher bite rate, fifteen times more loose calcareous matter was found to be produced by *C.striatus* inside the aquarium. Hence, an erosion rate of 0.09 mg per bite results for *C. striatus* compared to 0.001 mg/bite for *A. nigrofuscus*.

On all three reef sites both acanthurid species were very common and much more abundant than scarids (details of the surveys are omitted here).

The pooled data of bites per day of a single individual are 13679 (SD ± 2177) for *C. striatus* and 22220 (SD ± 5092) for *A. nigrofuscus*. Combining the experimentally obtained data of removed carbonate material per bite and observed number of bites on the reef per day results in the following hypothetical approximations of erosional efficiency: 449 g/ind.yr for *C. striatus* versus 6 g/ind.yr for *A. nigrofuscus.*

Table 1 Number of bites and amount of produced sediment [mg] per experimental run

A. nigrofuscus						
Individual A				Individual B		
Bites	mg	mg/bite		Bites	mg	mg/bite
27751	37.81	0.0014		13070	5.08	0.0004
22367	32.12	0.0014		6368	7.93	0.0013
C. striatus						
Individual A				Individual B		
Bites	mg	mg/bite		Bites	mg	mg/bite
5063	543.10	0.1073		1524	125.24	0.0822
3788	362.59	0.0957		3181	230.26	0.072

Discussion

The experiments resulted in a clear answer to the initial question which of the two acanthurids was responsible for the erosion of the *Favia* plates. A look at the mouthparts of the two species is helpful to understand the significant difference in the capability to remove carbonate substrate. The spatulate teeth of *A. nigrofuscus* are arranged in bundles (Fig 2) – well adapted to collect fine detrital material and algae. *C. striatus* is equipped with bristle-like teeth – well suited to sweep the bottom and to take in loose material (Fig. 3). In addition, there is a bow of hard knobs in the upper jaw (arrow in Fig. 3, SEM photo, Fig 4). This special palate structure is engaged to abrade hard bottom (Krone et al. 2006). This until recently overlooked morphological detail qualifies *C. striatus* as bioeroder – in addition to its affiliation to the guild of detritus feeders. The annual erosion impact of *C. striatus* is comparable to that of the sea urchin *Diadema setosum* in the Gulf of Aqaba which are eroding carbonate masses varying between 1023g/ind. (Kroll 1995) and 113g/ind. (Mokady et al. 1996).

On the basis of our estimates of abundance on some reefs *C. striatus* can achieve erosion rates of up to 70.0 g m^{-2} yr^{-1} which again reaches the same order of magnitude as that of the co-occuring sea urchin *Diadema setosum*. *A. nigrofuscus*, however, attains maximum erosion rates of 2.6 g m^{-2} yr^{-1} and may therefore not be regarded as a significant bioeroder.

The presented data and extrapolations are definitely conservative, since they were obtained from subadult fishes feeding on an especially hard substrate not yet weakened by endolithic borers (dried *Favia* plates were used to have standardized conditions with regard to the experiments mentioned in the introduction). Supplementary experiments run in the wet lab of the Ras Mohamed Park with natural reef substrate yielded five times higher excavation rates (unpub. data).

On the basis of the presented pilot study it might be fruitful to investigate the ecological functions of *C. striatus* within the sediment regimes of Indo-West-Pacific reefs in more detail.

Acknowledgement

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Differential effect of early post-settlement processes on the abundance of two concurrently settling coral reef fishes

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Abstract. Studies of early post-settlement processes in coral reef fishes have focused on small, sedentary species and have neglected the larger, more mobile, fished species. However, because recently settled fishes of both types are small and sedentary, early post-settlement processes might be similar. Here, we compare early post-settlement population dynamics of two naturally co-occurring taxa, *Sparisoma* (Scaridae), a poorly-studied medium-sized taxon important to artisanal fishers, and *Stegastes partitus* (Pomacentridae), a well-studied model species. We concurrently monitored density on three reefs in Barbados (West Indies) for 3-3.5 months following a period of high recruitment. Recruitment was >11x (*Sparisoma*) and >3x (*S. partitus*) greater than the initial number of large juveniles/adults. Recruit losses were very high (*Sparisoma* 97%; *S. partitus* 91%) for both taxa. However, the effect of recruitment on local population size differed qualitatively. After 3-3.5 months, *Sparisoma* recruitment had no effect on local juvenile density at any site, whereas *S. partitus* recruitment increased local density (by ~37%) at all sites. Our study does not support the applicability of small, model species to larger, fished ones, highlighting the need for more comparative studies.

Key words: Post-settlement, recruitment, settlement, mortality, reef fishes

Introduction

Most studies of population processes occurring within a few weeks after settlement in coral reef fishes involve a few model taxa, mainly pomacentrids, labrids and gobiids (Doherty 2002). These taxa are small, sedentary, abundant and conspicuous, offering multiple advantages as model species for ecological research (DeMartini 1998). In contrast, there are few studies of early post-settlement processes in the typically larger and more mobile species that are fished for food (Doherty 2002). It is important to know whether findings on the early post-settlement period of small, model species can be generalized.

Comparing early post-settlement population dynamics of co-occurring taxa can be particularly informative as recently settled fishes will be subject to the same environmental factors (e.g. Carr and Hixon 1995, Almany 2003). Although differences among taxa are expected, such comparisons will help reveal the magnitude and correlates of those differences.

During a year-round study on the west coast of Barbados, West Indies (Vallès et al. 2008), we identified a short concurrent period of peak recruitment in *Sparisoma* (Scaridae, likely *S. viride* + *aurofrenatum*, the two most abundant *Sparisoma* species at our sites) and *Stegastes partitus* (Pomacentridae). *Sparisoma* is a poorly-studied, medium-sized, fished taxon whereas *S. partitus* is a well-studied, small model species. This provided an opportunity to compare early post-settlement dynamics of two contrasting taxa at a time of naturally high recruitment.

Here we report the results from monitoring juvenile density of both taxa on three fringing reefs following the high recruitment period. We examine recruitment and subsequent loss rates and their ultimate effect on juvenile local population density over ~3.5 months. This is the period when both taxa are smallest and relatively sedentary, and thus, most likely to exhibit similar post-settlement effects (DeMartini 1998). We assess similarities and differences between taxa over the study period by asking: (1) what are the spatial and temporal patterns in recruitment?; (2) what is the magnitude of early post-settlement losses?; (3) what are the relative contributions of early post-settlement losses and recruitment to local density patterns?; and (4) what is the final effect of early post-settlement losses on local population density?

Materials and Methods

This study was carried out on three fringing reefs at least 2.5 km apart (Site 1: 13° 8′ 8′′N, 59° 38′ 22′′W; Site 2: 13° 11′ 48′′N, 59° 38′ 40′′W; Site 3: 13° 13′ 10′′N, 59° 38′ 41′′W), selected to capture the spatial variation in recruitment magnitude along the west coast (Vallès 2008). The habitat is a dense, patchy mixture of live and dead coral heads dominated by the star boulder coral *Montastrea annularis*, interspersed with sponges, sand and rubble. At each site, we established three permanent 6 x 6 m^2 quadrats at least 30 m apart for repeated surveys. We estimated the size of *Sparisoma* and *S. partitus* using a set of preserved specimens in a vial as a reference. We pooled the *Sparisoma* species because they are indistinguishable at first and are ecologically similar as small juveniles (Overtholtzer and Motta 1999). We allocated individuals to four non-overlapping size classes: recruits [*Sparisoma* <13mm standard length (SL); *S. partitus* <15mm SL], small juveniles (both taxa <20mm SL), medium juveniles (both taxa <30mm SL) and large juveniles/adults [*Sparisoma* <50mm SL; *S. partitus* >30mm SL, i.e. including all adults]. The upper size limit of the smallest size class (recruits) was set so that by the next survey, most recruits would have grown into the next size class (Vallès 2008). The largest size class was defined so that, by the end of the study, it included all the remaining fishes that originated from the observed recruitment. Because the recruit surveys were very time consuming, we sampled only one site per day and performed independent small juvenile surveys and large juvenile surveys. The small juvenile surveys took place every 15-d and involved recording the abundance of recruits, small and medium juveniles. The large juvenile surveys took place every 30-d and involved recording medium (again) and large juveniles/adults (Vallès 2008).

We estimated fish density over time as the mean number of individuals per quadrat (n = 3) at each site. We estimated cumulative recruitment as the sum of recruits recorded throughout the period of relatively high recruitment at each site. To assess the effect of recruitment on local density at the end of our study, we first estimated a "baseline" local density by combining the densities of the two largest size classes (medium and large juveniles) before the period of high recruitment. We calculated a "final" local density as the combined density of medium and large juveniles at the end of our study. We estimated per capita cumulative recruitment at a site by dividing cumulative recruitment by the "baseline" density.

For each taxon, we used two-factor repeated-measures ANOVAs to compare baseline and final local densities within and across sites and one-factor ANOVAs to compare cumulative recruitment across sites. Density data were square-root transformed prior to these analyses. Also, we used a two-factor repeated-measures ANOVA to assess whether recruit percent losses by the end of the study differed significantly across sites and between taxa. In all cases, quadrats were considered as random subjects and sites and taxa were considered as fixed factors.

Results

Recruitment of *Sparisoma* was similar in pattern and magnitude across all sites (Fig. 1). The bulk of recruitment occurred between the April 2 and July 2 surveys. During this period, there were two recruitment peaks at Site 1 and Site 2 during the May 2 and June 3 surveys, respectively, with the latter peak approximately 3 times as high as the former. A similar pattern was apparent at Site 3, except that the first peak merged into the second because of the asynchrony in sampling across sites. There was also substantial recruitment after July 3 at all sites, but it occurred too late to influence the juvenile density changes examined here and is not considered further.

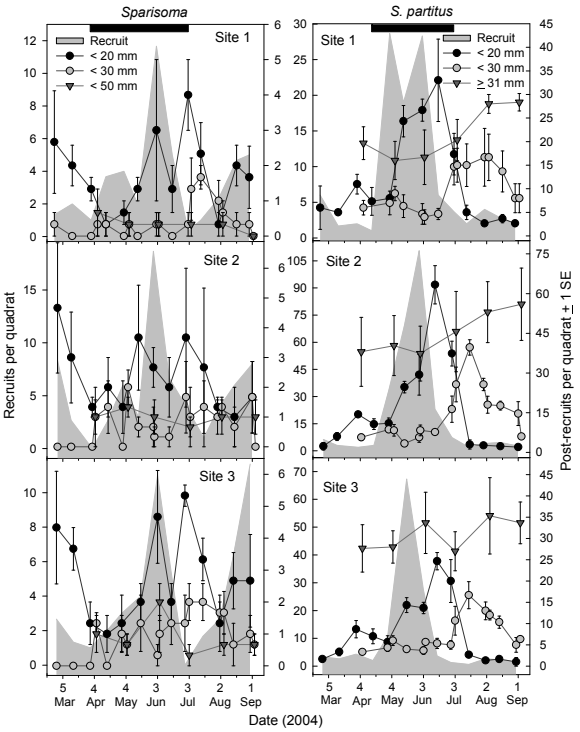

Figure 1. Average fish density on 3 permanent (36m^2) quadrats surveyed every 15-d for *Sparisoma* (left panels) and *S. partitus* (right panels) at three sites; the largest size class of both taxa was surveyed every 30-d. Separate lines represent 4 mutually exclusive size classes (mean ± 1 SE; for clarity, error bars for the recruit size class are not shown). Black horizontal bars indicate period of high recruitment monitored throughout the study.

For *Sparisoma*, recruitment pulses affected density in subsequent size classes, but were not

ultimately followed by increases in density at the end of the study. Increases in *Sparisoma* recruitment between April 2 and July 2 were followed by increases in small juvenile density (<20mm SL) at all sites (Fig. 1). Small juvenile density exhibited two apparent peaks, consistent with the two distinct previous recruitment peaks at Sites 1 and 2. There was substantial spatial variability associated with these juvenile density estimates, as indicated by the relatively large standard errors. Increases in small juvenile density were also followed by apparent increases in medium juveniles (<30 mm) at Sites 1 and 3 in the form of a single peak between July 3 and August 5. This peak was less apparent at Site 2. There was no indication of increases in large juveniles (<50 mm) at the end of the study. We found no significant evidence of an increase in final local density relative to the baseline and no significant differences among sites in local density (repeated measures ANOVA: time effect, $F = 2.520$, d.f. = 1, 6, $p = 0.164$; site effect, $F = 1.915$, d.f. = 2, 6, $p = 0.227$; time x site interaction, $F = 0.063$, d.f. = 2, 6, $p = 0.940$). In fact, final local density was slightly less than the baseline at all sites, resulting in negative net gains and percent increases at the end of the study (Table 1).

	Local density		
Taxon	Baseline	Final	% Increase
Sparisoma			
Site 1	1.0 ± 1.0	0.0 ± 0.0	-100.0
Site 2	2.0 ± 1.2	1.0 ± 0.6	-50.0
Site 3	2.3 ± 0.7	1.3 ± 0.7	-56.5
S. partitus			
Site 1	25.7 ± 2.0	36.3 ± 2.7	41.2
Site 2	43.7 ± 14.2	62.0 ± 13.9	41.9
Site 3	31.0 ± 5.5	40.0 ± 4.9	29.0
	Recruitment inputs		Recruitment
Taxon	Cumulative	Per capita	% losses
Sparisoma			
Site 1	27.7 ± 9.6	27.7	100.0 ± 0.0
Site 2	41.0 ± 23.1	20.5	96.4 ± 3.0
Site 3	26.3 ± 2.0	11.4	94.5 ± 2.7
S. partitus			
Site 1	86.7 ± 11.3	3.4	88.3 ± 5.1
Site 2	246.7 ± 51.1	5.6	92.5 ± 2.3
Site 3	112.3 ± 6.9	3.6	91.9 ± 0.8

Table 1. Above: local density of *Sparisoma* and *S. partitus* before a period of high recruitment (Baseline) and at the end of the study (Final, individuals per 36 m^2 reef quadrat ± 1 SE, N = 3) as well as the overall % increase in density. Below: cumulative recruitment (total recruits per 36 m^2 reef quadrat ± 1 SE, N = 3), per capita cumulative recruitment (cumulative recruitment divided by baseline) and percent of recruits lost by the end of the study.

Overall, cumulative recruitment over the entire April 2 – July 2 period did not differ significantly across sites (ANOVA: $F = 0.175$, d.f. = 2, 6, $p = 0.843$; Table 1), although per capita cumulative recruitment varied between 11.4 and 27.7 across sites; these latter estimates, however, are sensitive to small changes in local density because of the low baseline densities (Table 1). There was substantial variability in cumulative recruitment among quadrats within sites [average coefficient of variation within a site (CV): 56.9%].

For *S. partitus,* the bulk of recruitment occurred between the April 17 and Jul 2 surveys. There were differences among sites in the pattern and in the magnitude of recruitment (Fig. 1). At Site 1 recruitment was dominated by two distinct peaks of similar magnitude apparent in the May 2 and Jun 3 surveys. In contrast, at Sites 2 and 3, recruitment was dominated by a single large peak, apparent in the Jun 3 and May 18 surveys, respectively, possibly reflecting in part asynchrony in our surveys.

For *S. partitus*, increases in recruitment from April 17 to July 2 were sequentially followed by increases in densities of small juveniles (<20mm SL), medium juveniles (<30mm), and large juveniles/ adults near the end of the study at all sites (Fig. 1). Final local densities of *S. partitus* were significantly higher than baseline densities at all sites. There were no significant differences in local density among sites, and the magnitude of the increases did not differ across sites (repeated measures ANOVA: time effect, $F = 23.151$, d.f. = 1, 6, $p = 0.004$; site effect, $F = 1.938$, d.f. = 2, 6, $p = 0.224$; time x site interaction, $F = 0.505$, d.f. = 2, 6, $p = 0.627$, Table 1).

Overall, cumulative recruitment of *S. partitus* during the high recruitment period was more than two-fold higher at Site 2 than at the other sites (ANOVA: $F = 376.96$, d.f. = 2, 6, $p = 0.013$; Tukey test: Site 2 versus Sites 1 and 3, $p \leq 0.037$; Site 1 versus Site 3, $p = 0.667$, Table 1). Per capita cumulative recruitment varied between 3.4 and 5.6 across sites, and was at least 1.5-fold higher at Site 2 than at the other sites (Table 1). There was only moderate variability in cumulative recruitment among quadrats within a site (CV: 23.0%).

Estimated percent losses over the study period were high for both taxa, averaging 97.0% for *Sparisoma* and 90.9% for *S. partitus* across sites (Table 1). Percent losses differed significantly between taxa, but not across sites for either taxon (repeated measures ANOVA: taxon effect, $F = 5.966$, d.f. = 1, 6, $p = 0.05$; site effect, $F = 0.122$, d.f. = 2, 6, $p = 0.887$; taxon x site interaction, $F = 1.291$, d.f. = 2, 6, $p = 0.342$).

Discussion

In this study, we monitored density changes in two co-occurring taxa following a period of high recruitment. In doing so, we found both similarities

and differences between taxa in the effect of early post-settlement processes.

Similarities between taxa

Loss rates were consistently high for both taxa and most likely due to mortality. About 97% of *Sparisoma* and 91% of *S. partitus* recruits were lost from our sites 3-3.5 months after peak recruitment. Double-counting of recruits would have inflated such loss estimates; however, it is unlikely that this was a major problem because the recruit size classes were selected to allow most surviving recruits to attain the small juvenile size class before the next sampling date (Vallès 2008). Although we did not mark individuals and thus we cannot unequivocally distinguish losses due to migration and mortality, two lines of evidence suggest a negligible effect of migration. First, previous studies with marked juveniles within the size range of all those monitored in this study indicate average home range sizes considerably smaller than our quadrats (*Sparisoma*: Tolimieri 1998a, Fig 2 in Overtholzer and Motta 1999; *S. partitus*: Carr et al. 2002). Second, quadrats were placed on continuous reef so that movements in and out of the quadrats should have been balanced.

Post-settlement processes rather than recruitment variation appear to be the primary determinant of local population size for both taxa at our sites. Shulman and Ogden's (1987) sensitivity analysis of the relative effects of recruitment and post-settlement mortality on population size concluded that, at average mortality rates similar to those we observed, small changes in mortality would have a far greater effect on local juvenile population sizes than similar changes in recruitment.

We did not find significant site effects on post-settlement mortality for either taxon. However, this result warrants caution because spatial differences in post-settlement mortality of reef fishes over sites kilometers apart are common (e.g., Beukers and Jones 1997, Holbrook and Schmitt 2003). A parallel study suggested that the lack of a site effect reflected low power of our tests, given the small differences in average mortality across sites for both taxa (Vallès 2008).

Differences between taxa

The timing of recruitment peaks was generally consistent across sites for each taxon, but differed between taxa. This is consistent with the idea that settlement occurs synchronously coast-wide for each taxon in Barbados, but that temporal patterns in settlement differ between taxa (Vallès et al. 2008).

We found spatial differences in cumulative recruitment for *S. partitus* but not for *Sparisoma*. Differences in recruitment rates among sites a few kilometers apart are common (e.g, Booth et al. 2000). Here, they reflect spatial variability in cumulative settlement and/or mortality within our 15-d sampling intervals (e.g., Holbrook and Schmitt 2003). Vallès (2008) suggested that the habitat characteristics of Site 2, which differed from the other two sites, might have increased survival of *S. partitus* recruits. In contrast, the lack of spatial differences in *Sparisoma* recruitment may be due to low power associated with high within-site variability in recruit density for this taxon, compared to *S. partitus*. These differences in within-site variability suggest that relevant spatial scales of early post-settlement patterns and underlying processes (Sale 1998) differ fundamentally between these two taxa.

Cumulative recruitment over the study period was, on average, one order of magnitude higher for *S. partitus* than for *Sparisoma*. This was the case even though densities of both taxa were examined at the time of annual peak recruitment. Whether the lower *Sparisoma* recruit numbers reflect lower cumulative settlement and/or higher mortality between our 15-d surveys remains unresolved. This, however, should not affect our conclusion of significantly higher mortality for *Sparisoma* than *S. partitus* for the specimens observed over the study period.

Despite the lower number of *Sparisoma* recruits observed, per capita recruitment was considerably higher for *Sparisoma* (>11-fold) than for *S. partitus* (>3-fold). This difference was driven by the consistently low numbers of medium and large *Sparisoma* juveniles used as baseline density at our sites. Because larger size classes (>50 mm SL, including adults) of *Sparisoma* were similarly low at our sites (<2.6 ± 0.4 SE individuals per quadrat, unpublished data), including them in the baseline density would not have affected qualitatively our conclusion of substantially higher per capita recruitment for *Sparisoma* (>8-fold in the latter case).

The higher per capita recruitment of *Sparisoma* would have been expected to result in larger percent increases in local juvenile densities for this taxon, if early post-settlement processes were negligible or had a qualitatively similar effect across taxa. In contrast, the high mortality exhibited by *Sparisoma* ultimately resulted in a lack of increase in local density, whereas considerable increases (~37%) did occur for *S. partitus*. This pattern was very consistent across all sites, suggesting that our results are not an artefact of imprecise density estimates due to low counts or to insufficient quadrats per site. Thus, importantly, although mortality played a more significant role than recruitment in determining local juvenile density for both taxa, its ultimate effect on density varied qualitatively and fundamentally between taxa.

Although differences in early post-settlement mortality rates among juvenile coral reef fish species are common (Almany and Webster 2006), the mechanisms underlying such differences remain largely unexamined. In a concurrent study, Vallès (2008) suggested that differential availability of enemy-free space (Jeffries and Lawton 1984) contributes to the differences in magnitude and ultimate effect of mortality. In support of this, Vallès (2008) found that mortality of *Sparisoma* was considerably more strongly density-dependent than that of *S. partitus*, suggesting a lack of suitable refuge habitat against predators for the *Sparisoma* post-recruits at our sites (e.g., Forrester and Steele 2004).

Conclusion

The consistently low *Sparisoma* densities and the relative inconspicuousness of *Sparisoma* recruits may explain why there are few studies of recently settled *Sparisoma* (Tolimieri 1998 a,b, Paddack and Sponaugle 2008), despite the importance of this taxon in artisanal fisheries (Dalzell 1996) and reef processes (e.g., Mumby et al. 2006). Similar challenges likely explain the lack of studies on other recently settled scarids (e.g., Green 1992, Green 1998).

Bearing in mind the limitation of comparing only two taxa, we found only weak evidence that early post-settlement processes in a small model species can be applied to a larger, fished taxon studied at the same place and time. Although there were broad similarities between taxa, i.e. high mortality and high relative importance of post-settlement processes in determining local density, there were also major differences. In comparison to *S. partitus*, *Sparisoma* exhibited (1) higher spatial variability in recruitment over small scales, (2) higher per capita recruitment despite low absolute numbers of recruits, and, most importantly, (3) no increase in local densities only 3-3.5 months after a major recruitment pulse. This suggests fundamentally different local population dynamics soon after settlement. Thus, despite the challenges, our study demonstrates the need to further investigate early post-settlement processes in the larger and less numerically abundant coral reef taxa such as parrotfishes, whose abundance has important consequences for both reefs and fishers.

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Fish mesograzers as gatekeepers of the species composition of coral reefs

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Abstract. I present preliminary evidence that intermediate-sized (~15 cm long) fish mesograzers, principally parrotfish (scarids), play a critical role in controlling the species composition of coral reefs. I describe these mesograzers as gatekeepers because they control the entry of small (newly recruited) prey individuals into coral reef communities. The gatekeeper role of fish mesograzers is based on several well-recognized features of coral reefs: (1) Fish grazing has a major impact on abundances of benthic organisms; (2) Many species have a refuge in size from grazers; and (3) Artificial substrates in offshore habitats lacking fish mesograzers are often characterized by 'fouling' species that are absent in coral reefs. I hypothesize that only small individuals of fouling (and other) species are vulnerable to fish grazers because large prey individuals are protected by structural/chemical defenses. Because of their role as gatekeepers, the reduction of fish mesograzer populations by overfishing may have profound effects on the structure of coral reef communities.

Keywords: Gatekeeper, fish mesograzers, refuge in size, structural/chemical defenses

Introduction

In this report I propose that intermediate-sized (~15 cm long) fish mesograzers have a major impact on the structure of coral reef communities by feeding on small prey individuals. I describe fish mesograzers, principally parrotfish (scarids) and possibly surgeonfish (acanthurids) and other fish taxa, as 'gatekeepers' in the sense that they control the 'entry' (survival) of newly recruited prey into coral reef communities. This hypothesis is based on several well-recognized features of coral reefs.

First, numerous studies spanning several decades (e.g. Randall 1961; Valentine et al. 2007) have demonstrated that fish grazers can control abundances of prey species. In general, previous studies have focused on herbivory by fish grazers. For instance, Randall (1965) attributed the 'halos' surrounding Caribbean reefs largely to parrotfish grazing on seagrasses. Hay (1981) found that algal species such as *Botryocadia occidentalis* and *Spyridia aculeata* are excluded from Caribbean reefs by fish grazers. Hixon and Brostoff (1996) documented the effects of fish grazing on the succession of algal species. With some exceptions (Choat el al. 2004), parrotfish grazing on invertebrates have been largely ignored except for the direct effects on corals (e.g. Rotjan and Lewis 2006) or indirect effects involving overgrowth of corals by algae (e.g. Mumby et al. 2006).

Second, it is well known that many coral reef species possess a 'refuge in size' (Dayton 1971) from grazers and predators (Hughes and Connell 1987) due to structural and chemical defenses (Jackson and Buss 1975; Harvell and Fenical 1989). However, structural and chemical defenses may be ineffective against grazers when prey individuals are small and inconspicuous. Because macrograzers (large grazers) feed on larger prey (e.g. Bellwood et al. 2006), prey species with structural/chemical defenses may be grazed upon by mesograzers but not macrograzers.

Third, artificial substrates (buoy lines, mariculture cages etc.) are often heavily colonized by so-called fouling species, such as the octocoral *Carijoa* (*Telesto*) *riisei*, that are rare or absent in coral reefs (Humann 1993). Sutherland (1974) and others have previously demonstrated that fish grazing influences the structure of the fouling community on artificial substrates in marine systems. Thus, the presence of a coral reef fouling community suggests that fish grazing has a major impact on coral reef systems.

In this report I present preliminary evidence supporting the contention that the gatekeeper role of fish mesograzers structures coral reef communities. I focus on invertebrate fouling species to exemplify the effects of fish mesograzers. I also present results of pilot experiments that indicate small individuals of such species are especially vulnerable to fish mesograzers because they do not benefit from a refuge in size.

Methods and Results

This study was largely instigated by casual observations that the invertebrate epibiota on buoy lines used to locate benthic transects in nearshore, midshelf and shelf edge coral reefs off the southwest coast of Puerto Rico (18^{o} N, 67 ^{o} W) differed from species occurring on the reefs themselves. To confirm these impressions we surveyed the invertebrates on the buoy lines. Sessile invertebrates found on the buoy lines are listed in Table 1. Several species as the octocoral *Carijoa* (*Telesto*) *riisei*, the tunicate *Ascidea nigra*, and the anemone *Aiptasia tagetes* are regarded as coral reef fouling species, and are generally restricted to artificial substrates in coral reef habitats. Other species as the sponge *Dysidea janiae* and the wing oyster *Pteria colymbus* occur frequently in reefs but were more abundant on the buoy lines.

Table 1. Sessile invertebrate species found on buoy lines in nearshore, midshelf and shelf edge reefs near La Parguera, Puerto Rico. * generally absent in reef habitats.

SPONGES
Desmapsamma anchorata
Dysidea janiae
Monanchora barbadensis(?)
Mycale laevis
Mycale microsigmatosa
Tedania ignis
CNIDARIANS
*Aiptasia tagetes**
*Carijoa riisei**
Halocordyle disticha
MOLLUSCS
Pinctada radiata
Pteria colymbus
Dendrosta frons
TUNICATES
*Ascidea nigra**
Botrylloides nigrum
Didemnum sp
Polycarpa spongiabilis
Symplegma viride(?)

Vertical patterns of distribution indicated that fish grazing was responsible for the presence and abundance of the epibiotic species occurring on the buoy lines. At the nearshore and midshelf reefs, only portions of buoy lines more than ~1.5 m above the substratum were colonized by sessile organisms. In contrast, epibiotic cover was low on all portions of the buoy lines at the shelf edge reefs. These vertical patterns coincided with the distribution of fish grazers. The black durgon, *Melichthys niger*, was restricted to the shelf edge reefs, and was observed feeding throughout the water column. Alternatively, parrotfish mesograzers, principally the redband parrotfish *Sparisoma aurofrenatum*, striped parrotfish *Scarus croicensis* and stoplight parrotfish *Sparisoma viride* occurred at all sites but always remained close to the bottom presumably because of the cover provided from piscivores.

As pilot experiments to test whether fish grazers were responsible for the vertical patterns of the epibiota, we lowered the upper portions of buoy lines to within about a meter of the bottom at two midshelf reefs. Almost immediately, parrotfish, wrasses, surgeonfish and butterfly fish began grazing on the buoy lines (Fig 1a). After about a week, abundances of most epibiota, especially algae, were severely reduced on the lowered portions of the buoy lines except for a colony of the sponge *Dysidea* (Fig. 1b). *Dysidea* is evidently defended chemically because a redband parrotfish *Sparisoma aurofrenatum* spit out *Dysidea* fragments soon after grazing it (Fig 1c). Hay (1991) noted that parrotfish often taste an unfamiliar item before rejecting it. Interestingly, *Sparisoma aurofrenatum* and other Caribbean parrotfish mesograzers are generally regarded as herbivores (e.g. Bruggemann et al. 1994) based on gut contents (e.g. Randall 1967). These observations indicate that various invertebrate species may be grazed upon, but not ingested, by meso-sized parrotfish. Thus, these parrotfish are probably more appropriately regarded as omnivores rather than herbivores in terms of their grazing activities.

An analogous situation with *Dysidea* occurred with a *Carijoa* colony on another lowered buoy line (Fig. 2). These results indicate that the *Dysidea* and *Carijoa* colonies had reached a refuge in size from grazers. By extension, other species occurring on the buoy lines may also benefit from a refuge in size from fish grazers.

Discussion

The maximum time that the buoy lines were on the reefs was about 3 years. This raises the alternative interpretation that the species observed in this study simply represent the initial colonizing stages of species succession, which over time will develop into a climax community dominated by scleractinian corals and other taxa. However, species such as *Carijoa* have not colonized reefs in the study area following hurricanes (Yoshioka and Yoshioka 1987) or other disturbances (pers. obs.). Additional considerations indicate that the short time span of this study is not necessarily the critical factor. For example scleractinian corals are absent on >40 year-old pier pilings locate in a sand plain habitat near Aguadilla, Puerto Rico where fish mesograzers are

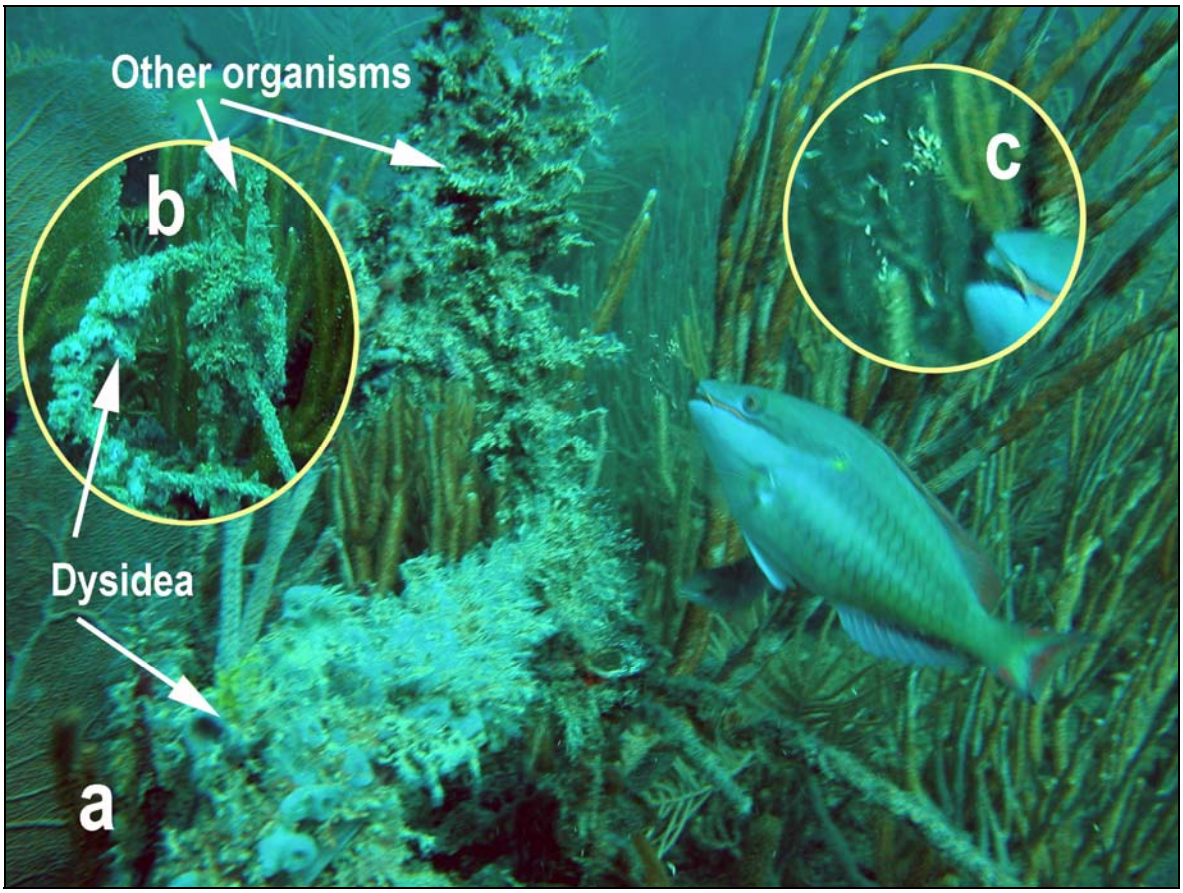

Figure 1. Fish grazing on a lowered buoy line. (a) Grazing by a redband parrotfish (*Sparisoma aurofrenatum*). (b) A *Dysidea janiae* sponge colony and other biota a week later. (c) The *S. aurofrenatum* rejecting fragments of the *Dysidea* colony.

absent (pers. obs.). Alternatively scleractinians are relatively abundant on 30 year-old metal bars in a shelf edge reef site in the study area where fish mesograzers are abundant (pers. obs.). These observations concur with the Inhibition Model of succession (Connell and Slayter 1977) wherein grazing may alter the course of succession.

With respect to terms used to describe ecologically important species, gatekeepers can viewed as a modification of the keystone species concept emphasizing prey size. Paine (1966) introduced the keystone species concept with the starfish *Pisaster ochraceus*, which controls 'post entry' abundances of the mussel *Mytilus californianus* by feeding on large (mature) individuals. In contrast gatekeepers prevent the 'entry' of prey populations into communities by feeding on small (newly settled) individuals. The emphasis on prey size is especially important if prey individuals enjoy an escape in size from grazers and predators. Paine (1976) recognized the importance of prey size by demonstrating that some *Mytilus* may grow too large for consumption by *Pisaster*.

In terms of fundamental issues in population dynamics, the gatekeeper scenario is consistent with results indicating that mortality during the early benthic life stage may be the key factor controlling variations in abundances of coral reef organisms (e.g. Yoshioka 1997). Because newly recruited prey individuals are small, the biomass of such individuals is a poor indicator of the ability of gatekeepers to control prey populations. Mumby et al. (2006) concluded that large parrotfish are more effective than meso-sized parrotfish in controlling macroalgae because of the disproportionately greater amount of biomass consumed. The gatekeeper scenario presents the alternative argument that grazing by meso-sized parrotfish may be more effective in controlling macroalgae by grazing on large numbers of small plants.

To my knowledge the gatekeeper role of fish mesograzers has received little attention in coral reef systems. In contrast, gatekeeper effects are relatively well documented in terrestrial systems. Tansley and Adamson (1925) showed that grazing by rabbits prevents the entry of shrubs and trees in grasslands of England. Ripple and Beschta (2008) demonstrated that deer grazing has prevented the successful recruitment of black oak trees for over 80 years in

Figure 2. A *Carijoa riisei* colony on a lowered buoy line. (a) Before gatekeeper grazing. (b) A week after gatekeeper grazing.

some areas of Yosemite Park. The gatekeeper scenario is reminiscent of the hypotheses of Janzen (1970) and Connell (1971) that grazing on seeds or seedlings in the vicinity of parent trees is a major factor in the maintenance of biodiversity in tropical rain forests. However, Janzen (1970) and Connell (1971) emphasize the species-specific preferences of grazers. In contrast the gatekeeper scenario proposed for coral reefs emphasizes nonselective grazing by fish mesograzers. Because the effects on nonselective grazing are more widely distributed among many prey species, relatively high levels of nonselective grazing are probably required for equivalent effects on a given prey species compared to selective grazing. This is evidently the case. Randall (1961) concluded that fish can 'overgraze' reefs, as exemplified by bare sand halos surrounding patch reefs (Randall 1965). Hay (1991) asserted that fish grazing on coral reefs equals or exceeds grazing rates for any other marine or terrestrial system. Fox and Bellwood (2008) estimated that the parrotfish *Scarus rivulatus* grazes >50% of the reef substratum on a monthly basis.

It must be emphasized that the gatekeeper scenario outlined in this report is a simplified overview of the role of fish mesograzers in coral reef systems. Many species-specific features of grazer and prey species merit attention. For example, depending upon the species involved, there is probably a sequential series of refuges in sizes from grazers (Paine 1976) rather than a single threshold in size. Assuming that structural/chemical defenses are equally effective against grazers, faster growing prey species may reach a refuge in size more quickly. Alternatively, faster growth may occur at a cost of allocating less metabolic resources to structural and chemical defenses (Paine 1977). Gatekeeper effects are probably also contingent on prey morphology. Grazing is more likely to be fatal for solitary compared to colonial organisms since damage often results in 'partial mortality' of the latter and 'complete mortality' for the former (e.g. Harvell and Suchanek 1987). Hay's (1991) observation that parrotfish taste defended prey before rejecting it is noteworthy in this regard because such taste tests may be fatal for solitary, but not colonial, organisms. Also, encrusting forms are less vulnerable than erect forms because substrate scraping by fish mesograzers is more likely to remove erect organisms. Similarly, multi-stemmed erect organisms are less vulnerable than single stemmed individuals.

Finally, I stress that, in addition to grazer-prey interactions, the gatekeeper scenario outlined herein also overlooks grazing by invertebrates and competition among prey species. Anthropogenic influences cannot be ignored because the tendency of 'fishing down the food chain' (Pauly et al. 1998) may severely reduce fish mesograzer abundances in many areas. Because the direct and indirect effects of gatekeeper grazing are largely unknown, the over harvesting of fish mesograzers may result in unforeseen and profound changes in the structure of coral reef systems.

Acknowledgements
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Comparison of Sedimentation in Bays and Reefs below Developed versus Undeveloped Watersheds on St. John, US Virgin Islands

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Abstract. Increased terrigenous runoff associated with building and development is one of the most serious threats to coral reefs. Here we evaluate how the sediment flux rates and compositions (carbonate, terrigneous [siliceous], and organic matter) differ between reefs and bays below a developed watershed compared to undeveloped watersheds on St. John, US Virgin Islands. Detailed monitoring of sedimentological processes affecting individual reefs is a critical compliment to ecological monitoring and necessary to evaluate the effectiveness of erosion mitigation strategies. During the 2007 rainy season, sediment flux rates were at least 3 and up to 73 times higher on the reefs below a developed watershed compared to reefs below an undeveloped watershed. The developed watershed sediment flux rates (3-630 mg $cm^{-2}d^{-1}$) were sometimes within range of rates previously shown to cause "severe to catastrophic" sediment stress to corals. Mean seasonal terrigenous flux rates were 15 times higher (5 vs. 0.3 mg $cm^{-2}d^{-1}$) and mean organic matter flux rates were 10 times higher (3 vs. 0.3 mg $cm^{-2}d^{-1}$) on the reefs below the developed watershed.

Key words: sedimentation, sediment flux, Virgin Islands, carbonate, TSS

Introduction

Sedimentation is one of the primary causes of coral reef degradation in the US Virgin Islands (Rogers 1990; Rogers 1998). Sedimentation stress to corals depends on the quantity, duration, and composition of sedimentation (Fabricius 2005; Philipp and Fabricius 2003; Riegl and Branch 1995). In order to effectively mitigate the potentially detrimental impacts of sedimentation to a reef, it is critical to determine the rate, mechanism, and quality (type) of sediment flux into a particular reef system.

Here we present preliminary data from an ongoing study to evaluate if development in St. John, US Virgin Islands (Fig. 1) has impacted the quantity, quality, and spatial variability of sedimentation in four bays with fringing reefs—one below a developed watershed (Fish Bay; Fig. 2A) and three below undeveloped watersheds located within the Virgin Islands National Park (VINP) (Great Lameshur, Reef, and Hawksnest Bays; Fig. 2 B-E). We addressed the following research questions: 1. What is the composition and quantity of suspended, settling (sediment trap), and accumulated (bay-floor bottom) sediments? 2. How does the sediment composition and quantity: a) vary spatially within each bay along pathways of sediment dispersal and among the bays? b) vary temporally during the study period (August-November, 2007); and c) vary between the developed and undeveloped bays? St. John, USVI is an ideal location to study the impacts of sedimentation on coral reefs because: a) there is a distinct delineation between developed and undeveloped watersheds due to the VINP (Fig. 1); b) sediment originating from land (terrigenous) is compositionally distinct (siliceous) from marine (carbonate) sediment due to the lack of terrestrial carbonate sources; and c) the fringing reefs on the island are well monitored (Rogers 1998; Rogers and Miller 2006; Edmunds 2005).

On St. John, development and construction activities outside of the VINP boundaries have brought shallow septic systems, dirt roads and impervious surfaces with increased erosion from roadbeds and cut slopes. Previous studies established a comprehensive sedimentation budget for the land area of St. John and established that there is a high "sediment delivery potential" to Fish Bay below its developed watershed (Ramos-Scharrón and MacDonald 2005; MacDonald et al. 2001, Nemeth et al. 2001).

Material and Methods

Sediment trap transects generally followed sediment runoff dispersal routes from guts (seasonal streams) into bays and across fringing reefs (Fig. 2). Bay-floor bottom sediment (upper 3 cm) transects closely replicated the bottom sediment sampling locations of Hubbard et al. (1987) in Fish, Reef and Hawksnest Bays (Fig. 2).

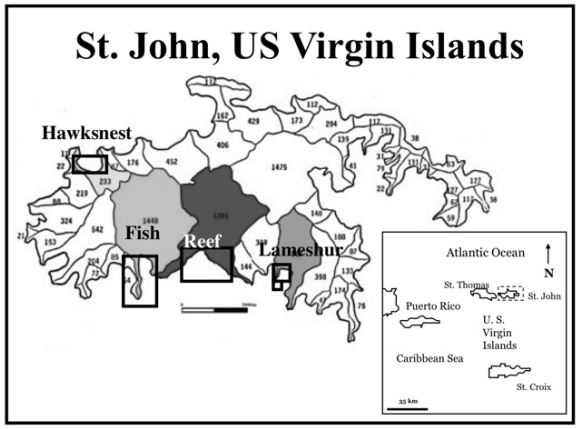
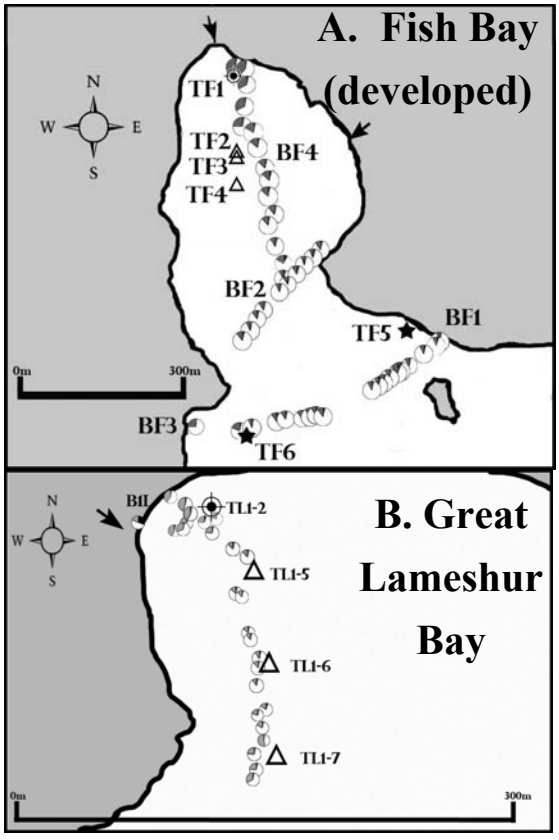


Figure 1: Map of St. John the four watersheds draining into the four study areas: Hawksnest Bay, Fish Bay, Reef Bay, and Great Lameshur Bay. Most of Fish Bay watershed is developed the other three watersheds are within the boundaries of the Virgin Islands National Park and thus considered undeveloped watersheds. The rectangles mark the locations of detailed maps (Fig. 2). Figure modified from Hubbard et al. (1987).



The variations in settling sediment (flux rates) were determined by deploying 14 sediment trap arrays (each with 4 exchangeable PVC tubes [length:diameter = 4:1]) attached to a metal stake 60 cm above the bay floor at 1-8 meters water depth in Fish and Great Lameshur Bays (Figs. 2A, 2B, 2C).

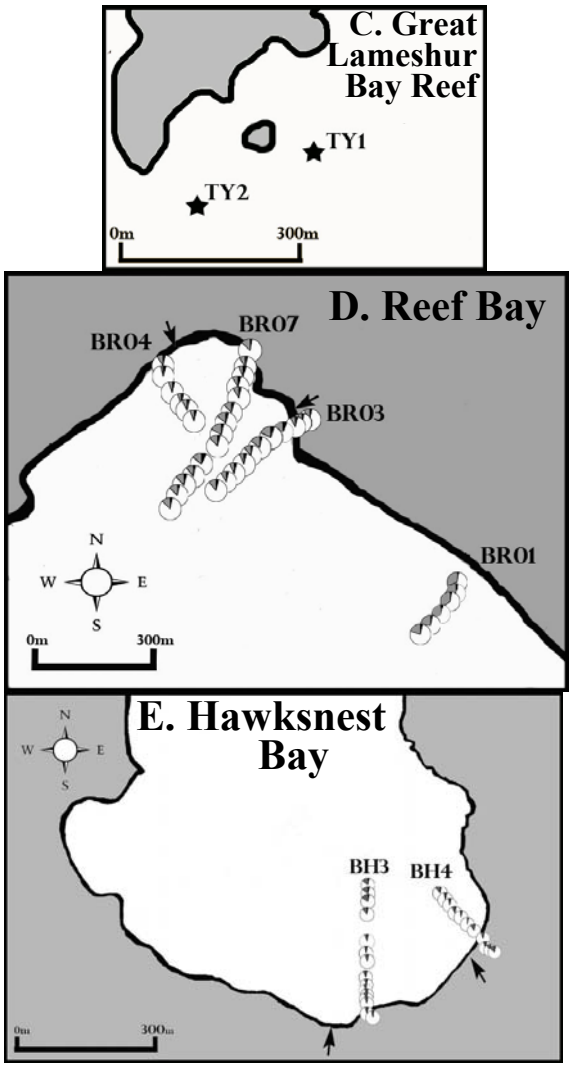


Figure 2: Maps of St. John bays showing sampling location and bay-floor bottom sediment compositions for A) Fish Bay (developed bay), B) Great Lameshur Bay, C) Yawzi Point, D) Reef Bay and E) Hawksnest Bay. Targets, triangles, and star symbols indicate the locations of sediment traps in shore, bay, and reef locations, respectively for Fish Bay (A: TF1 through TF6) and Great Lameshur Bay (B: TL1-2 through TL1-7) and Yawzi Reef (C: TY1, TY2). Pie diagrams indicate the locationsof surface bottom sampling transects in Fish (A: BF1 through BF-4), Great Lameshur (B: B1L), Reef (C: BR01, BR03, BR04, BR07) and Hawksnest (D: BH3, BH4) bays. Arrows mark the locations of guts (seasonal streams). The pie diagrams show the relative proportions of carbonate (white), terrigenous (grey) and organic matter (black) in each bottom surface sediment sample.

Sediment accumulation was monitored after 8 sampling periods (typically 12-14 days) between August-December of 2007. Sediment traps were collected and deployed at all locations within a six-hour time period. Sediment trap sediments were filtered, rinsed, dried and weighed to provide replicate measurements of the dry mass of sediment flux over the time deployed. Water samples were collected next

to each sediment trap and at the surface when sediment traps were changed (non-storm days) and filtered through a pre-combusted glass fiber filter (GF/F Whatman) to determine the total suspended sediment. The % organic matter and carbonate were determined by Loss on Ignition (LOI) at 550°C and 950° C, respectively following techniques modified from Henri et al. (2001). Additionally, a subset of samples was sieved to determine the variation in mineral composition with grain size.

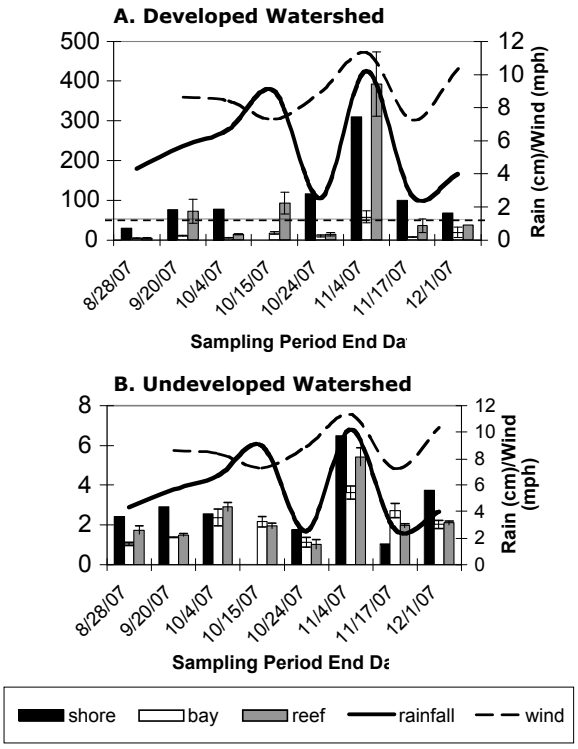


Figure 3: Variation in total sediment flux with time (Fall 2007), with total rain (cm) and mean wind speed for each sampling period in the A) developed and B) undeveloped watershed. The dashed line marks a flux rate of 50 mg $cm^{-2}d^{-1}$ above which may be detrimental to corals.

Table 1: Ratio of unsieved sediment flux below the developed watershed (Fish Bay) to unseived sediment flux below the undeveloped watershed (G. Lameshur Bay)

Sampling Period	28-Aug	20-Sep	4-Oct	15-Oct	24-Oct	4-Nov	17-Nov	1-Dec
Shore	12	26	30		66	48	94	18
Bay	4	8	2	8	8	16	3	9
Reef	3	48	5	48	14	73	18	18
Whole bay	**6**	**25**	**8**	**18**	**24**	**45**	**15**	**12**

Results

Unsieved sediment flux rates ranged from less than 1 to 400 mg $cm^{-2}d^{-1}$ (Fig. 3). The greatest sediment flux rates occurred between 10/25/07-11/4/07 when rain and mean wind speeds were highest (Fig. 3). Total mean sediment flux rates for the shore, bay, and reef stations were significantly higher in the developed

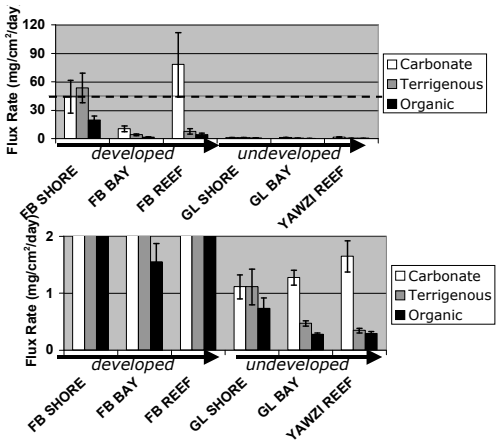


Figure 4: Mean seasonal flux rates for carbonate, terrigenous (siliceous) and organic matter were many times higher below the developed (Fish Bay) compared to the undeveloped (G. Lameshur Bay) watersheds. Arrows point from near to offshore stations. Upper and lower graphs are the same but with different vertical scales (FB: Fish Bay; GL: Great Lameshur; Yawzi Reef is in G. Lameshur Bay). The dotted line in the upper graph marks a flux rate of 50 mg $cm^{-2}d^{-1}$ above which may be detrimental to corals.

(Fish) compared to the undeveloped bay (G. Lameshur Bay) (p values = 0.0018, 0.0003, and 0.0109 for the shore, bay and reef, respectively)(Fig. 3). Compared to the flux rate at the undeveloped stations, mean sediment flux rates were up to 73, 16, and 94 times higher on the developed reef, bay and shore stations, respectively (Table 1).

The mineralogic composition of the sediments did not appear to vary much between grain size fractions for either the bottom or sediment trap samples. Carbonate, terrigenous and organic matter was found in all grain size fractions suggesting that sieving is not an appropriate way to separate terrigenous from carbonate constituents.

Carbonate was the most abundant constituent at most sediment trap stations except those nearest the shore (Fig. 4). Mean seasonal carbonate, terrigenous, and organic flux rates were up to 110, 128 and 28 times higher, respectively at the stations below the developed compared to the undeveloped watershed (Fig. 4). However, a pattern of decreasing terrigenous with increasing carbonate sedimentation with distance offshore was evident at both locations (Fig. 4).

Distinct compositional sediment flux ratios characterized sediments collected in each of the three environments (shore, bay, reef) (Fig. 5). Although the compositional flux ratios from the developed and undeveloped stations overlap, the flux ratios from the developed bay (Fish Bay) show a much wider range of values than do those from the undeveloped bay (G. Lameshur Bay) (Fig. 5).

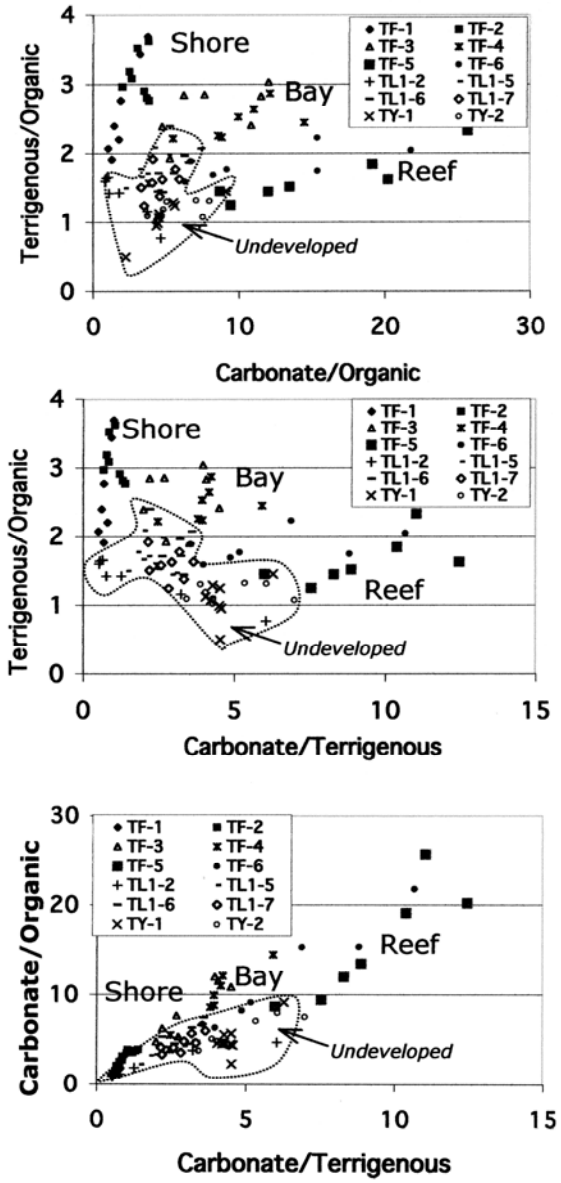


Figure 5: Distribution of compositional flux rate ratios for each sediment trap during each of 7 sampling periods indicated in Figure 3. The distribution shows distinct ratios for the reef, bay and shore environments, but a wider range of values for the developed compared to the undeveloped (within dotted lines) stations.

Like the sediment trap samples, the bay-floor bottom sediments (upper 3 cm) were comprised predominantly of carbonate, followed in abundance by terrigenous and organic matter (Fig. 6).The relative % carbonate and the ratio of carbonate to terrigenous (C_i/T) material in the bottom sediments increased with distance from the shore to the reef in both Fish and Great Lameshur Bays but not in Reef or Hawksnest Bays (Fig. 6). The percentage organic matter differed between the three sediment reservoirs (suspended: 36%-68%; trapped: 6%-23% and bottom: 2% to 4%) (Fig. 7). Mean total suspended sediment

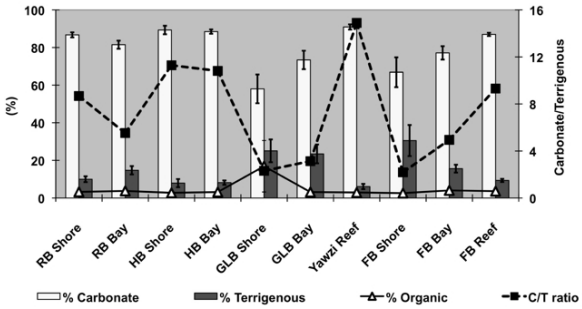


Figure 6: Composition of bottom samples (in %). The solid line marks organic matter % and the dashed line marks Carbonate/Terrigenous ratios (Ci/T). (RB: Reef Bay; HB: Hawksnest Bay; LB: Great Lameshur Bay; FB: Fish Bay).

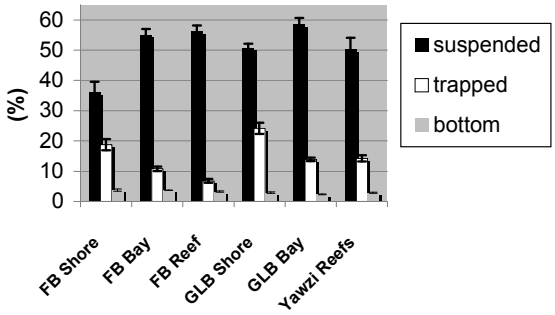


Figure 7: Mean % organic matter in three sediment reservoirs (suspended, trapped & bottom) (FB: Fish Bay; GLB: Great Lameshur Bay).

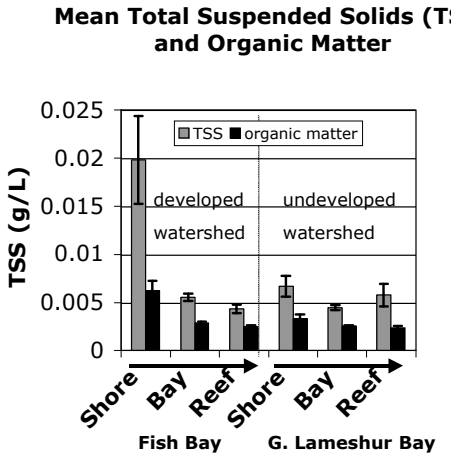


Figure 8: Mean total suspended solids (TSS) and suspended organic matter for shore, bay and reef locations below the developed and undeveloped watersheds. Arrows point from near to offshore stations. TSS and suspended organic matter were significantly higher at the shore below the developed watershed.

for the season ranged from 0.004 to 0.02 g/L and was significantly higher at the developed compared to the undeveloped shore and bay stations (p values = 0.0114 and 0.0151, respectively) (Fig. 8). The concentration of suspended organic matter was

significantly higher in the developed compared to the undeveloped bays ($p = 0.0005$).

Discussion

What can account for an up to 73-fold higher total flux and an up to 128-fold higher terrigenous flux on the Fish Bay compared to the Great Lameshur Bay (Yawzi) reefs? This difference is too large to be explained by the fact that the area of the Fish Bay watershed is three times the area of the Great Lameshur watershed. The Fish and Great Lameshur Bay watersheds have similar mean slopes, soil types, and vegetative cover. The bays are of generally similar geometry, orientation, and exposure to swells, tides and currents. The fact that the composition of the sediments in the traps is primarily carbonate suggests that the traps are likely collecting sediments that have been resuspended by waves and currents in addition to terrigenous sediments delivered directly in suspension from terrestrial runoff. There is greater availability of terrigenous sediment in Fish Bay (developed) compared to Great Lameshur Bay (undeveloped). We are conducting further studies to evaluate the sediment availability to resuspension and have deployed current meters to quantify the specific relationship between current velocity and sediment flux into the traps.

These data clearly show that the corals at Fish Bay reef are under significantly greater sediment stress than the corals at Great Lameshur (Yawzi) reef. The total sediment flux rates at Great Lameshur Bay reef were consistent with rates previously recorded for reefs that were not subjected to human activities (<1-10 mg $cm^{-2}d^{-1}$: Rogers, 1990). However, the sediment flux rates on the Fish Bay reef were high enough to be potentially dangerous to the reefs. Pastorak and Bilyard (1985) suggested that flux rates of 10-50 mg $cm^{-2}d^{-1}$ and > 50 mg $cm^{-2}d^{-1}$ could cause "moderate to severe" and "severe to catastrophic" sediment stress, respectively. Sediment flux rates on Fish Bay reefs were higher than 50 mg $cm^{-2}d^{-1}$ during three of the 8 sampling periods and above 300 mg $cm^{-2}d^{-1}$ during one. These high flux rates (>100 mg $cm^{-2}d^{-1}$) have been shown to kill exposed coral tissue within a few days (Riegl and Branch 1995) or reduce photosynthetic yields (Philipp and Fabricius 2003). Studies suggest that organic sediment may be particularly harmful to corals (Fabricius 2005). The reef below the developed watershed had organic flux rates up to 28 times higher than the reef below the undeveloped watershed.

Though bay-to-bay flux rate differences were quite dramatic, compositional differences between fluxing sediments were more subtle and were revealed by examining within-bay and bay-to-bay differences in the relative ratios of constituent flux rates.

Comparisons of both quantity and compositions of the suspended and bay floor bottom sediments did not reveal striking differences between the developed and the undeveloped locations. Our preliminary comparisons show that the composition of bottom sediments has not systematically changed (within 5% relative %) since the Hubbard et al. (1987) sediment survey despite intensive development in the area. Continued research through the 2008 season is underway as well as textural and geochemical analyses of the 2007 sediments.

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PROCEEDINGS OF THE

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From Molecules to Moonbeams: How is Reproductive Timing Regulated in Coral Reef Organisms?

Convened and edited by:

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Spatial patterns of reproductive synchrony by four genera of tropical green seaweed across a latitudinal gradient in the Caribbean.

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Abstract. Spatial and temporal patterns of reproductive effort are an especially significant feature of fertilization success for virtually all broadcast spawning organisms. While most research efforts on this topic have focused on varying temporal scales of reproductive synchrony (i.e., diel, lunar, and seasonal scales), patterns of reproductive synchrony on varying spatial scales (i.e. from meters to hundreds of kilometers) are less well studied. Simultaneous investigations of sexual reproduction by tropical green seaweeds (e.g., *Caulerpa, Halimeda, Penicillus, Udotea*) across a latitudinal gradient in the Caribbean (Panama, U.S. Virgin Islands, Florida) reveal patterns of reproductive synchrony on different spatial scales. Within a region, spatial synchrony often extends beyond local populations (meters to tens of meters) to at least scales of tens of kilometers, however, such synchrony is not maintained across larger (hundreds of kilometer) spatial scales. Relative synchrony within and between genera is maintained across this larger latitudinal gradient, however. Reproductive seasonality also shows an interaction with latitude for these seaweeds, with shorter, temporally delayed seasons of reproductive activity occurring at higher latitudes.

Key words: Broadcast spawning, Coral reef, Green algae, *Halimeda*, *Penicillus, Udotea*.

Introduction

Broadcast spawning, the release of gametes directly into the water column, is a common reproductive tactic for a diverse array of benthic marine organisms including stony corals (e.g., Harrison *et. al*. 1984; Baird et. al., 2001), soft corals (e.g., Lasker et al. 1996), echinoderms (e.g., Babcock and Mundy 1992; Levitan 1991, 1995), green algae (e.g., Clifton 1997, Clifton and Clifton 1998), sponges (e.g., Hoppe and Reichert 1987; Fell 1993; Ritson-Williams et al. 2005), and mollusks (e.g., Shelley and Southgate 1988; Lucas 1994; Kenchington et al. 2006). Synchrony of gamete release in both space and time is a consistent feature of these reproductive events, as organisms that match the timing of their gamete release with those of neighbors should enjoy increased fertilization success. Understanding the proximate cues that organize these reproductive events, as well as their ecological and evolutionary consequences remains a topic of interest to coral reef biologists.

Broadcast spawning can be examined at diel, lunar, and seasonal temporal scales. Patterns of reproductive timing are becoming increasingly better known, particularly for well-studied broadcast spawners like the stony corals (e.g., Tanner 1996, Guest 2004, Guest et al., 2005, Mangubhi and Harrison 2008). Studies of spatial patterns of reproduction have received less attention (although see: Oliver et al. 1988; Willis et al. 1985; Hughes et al. 2000; and Baird et al. 2002). As might be expected, even less is currently known about the degree to which temporal patterns of broadcast spawning behavior are preserved or altered at increasingly larger spatial scales.

This paper describes temporal and spatial patterns of broadcast spawning behavior for 4 genera of Caribbean seaweed (Bryopsidales) across a latitudinal gradient of 15°. Diel and seasonal patterns of gamete release for these algae at a single location (Caribbean Panama) are already described (Clifton and Clifton 1998). When compared with more northerly sites (St Croix, US Virgin Islands and Key Largo, Florida), changes in the diel timing of gamete release shifted with changes in the onset of sunrise. At the most northern site seasonal shifts in reproductive activity correlated with an annual change in water temperatures. Interspecific patterns of reproduction observed at local spatial scales were preserved over latitudinal gradients. This suggests that, at any one location, the relative contribution that various endogenous and environmental factors ultimately make to the timing of reproduction will vary, depending on the temporal scale being examined.

Material and Methods

Four genera of green macroalgae in the Order Bryopsidales (*Halimeda*, *Caulerpa*, *Penicillus*, and

Udotea) were observed for this study. All are common and abundant within shallow tropical marine communities across the Caribbean, including coral reef, seagrass bed, and mangrove habitat. Algae were identified to species using Littler and Littler (2000) and Littler *et. al.,* (1989).

Reproductive activity was quantified from daily surveys of shallow marine habitats at one of three sites: Kuna Yala, Panama (9°33' N, 78°56'W); Tague Bay, St Croix, US Virgin Islands (17°45' N, 64°36'W; and Key Largo, Florida (25°05' N, 80°24'W). Observers snorkeled along proscribed routes for 30–60 minutes, examining a minimum of 500 thalli for macroscopic evidence of impending sexual reproduction. This occurs 12–24 hrs prior to gamete release for seaweeds in the genus *Halimeda* and *Caulerpa*, 36–48 hrs for *Penicillus* and *Rhypocephalus*, and 60-72 hrs for *Udotea* (Clifton and Clifton 1998). Time of gamete release was recorded during direct observations of undisturbed seaweeds during the early morning spawning period. Initial time and duration of gamete release was noted following the methods of Clifton and Clifton (1998). Seawater temperatures for Panama and St Croix were measured using "StowAway Tidbit" automated temperature loggers (Onset Computer Corp). Temperatures from Florida were downloaded from archived NOAA Data Buoy information (www.ndbc.noaa.gov).

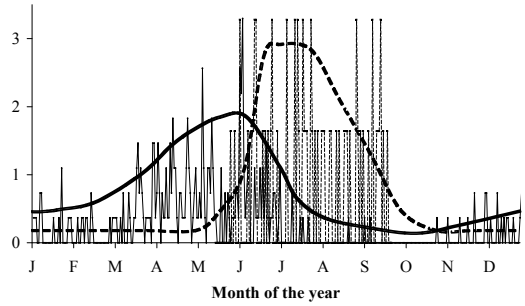

Figure 1: Seasonal patterns of sexual reproduction by green seaweeds at two sites: Panama (solid lines) and Florida (dashed lines).

Results

Seasonal patterns of reproduction

Populations of tropical green macroalgae across the Caribbean showed different seasonal patterns of reproductive activity. At 9° N latitude (Panama), reproduction occurred almost year-round, with a broadly seasonal peak from April to July that roughly corresponds to the onset of the region's annual wet season (Fig. 1). At 15° N latitude (Florida), reproductive activity occurred later in the year, between the months of June and September (Fig. 1). In general, a larger proportion of the population participated in a reproductive event on any given day at the more northern site (K. Clifton, *unpub data*), suggesting that, on an annual basis, total levels of reproduction between the two sites may not be that different. The seasonal peak of reproduction at the northern site correlated with the period of time when local water temperatures exceeded 27°C (Fig. 2).

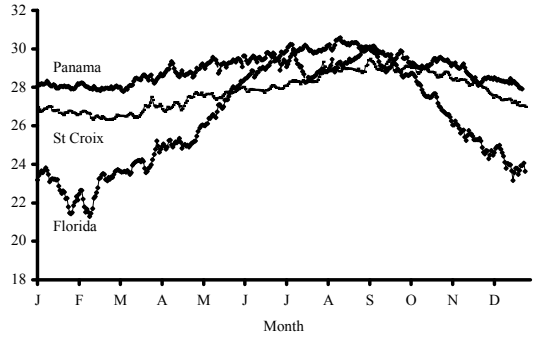

Figure 2: Seasonal variance in surface temperature at three Caribbean sites. The period of time when temperatures exceed 27°C is shorter and later in the year at higher latitudes. See text for details.

Sub-seasonal patterns of reproduction

The sub-seasonal scheduling of sexual reproduction by tropical green seaweeds does not show any obvious relationship to lunar or tidal cycles (Clifton 1997). There is, however, some regularity of spawning effort and this differs from one species to the next. For some species, a subset of the population reproduces every few days and this pattern is maintained across sites that differ in latitude. For example, the alga *Caulerpa cupressoides* reproduced roughly once every 7 days during the seasonal peak of reproduction in 1999 at all sites (Fig. 3).

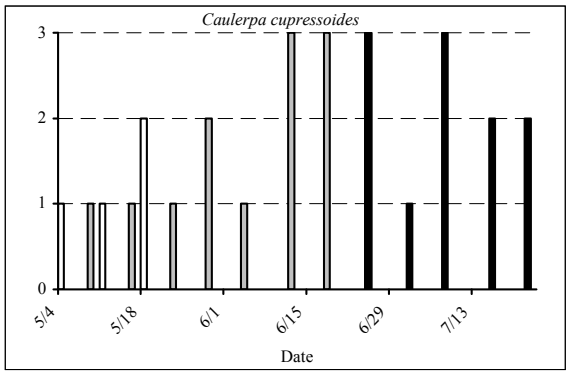

Figure 3: Patterns of sub-seasonal reproduction for *Caulerpa cupressoides* at three Caribbean sites. Color scheme as follows: open bars = Panama; shaded = St. Croix, USVI; black = Key Largo, Florida. "Extent of population" represents the percent of the population participating in the reproductive event as follows: 1: < 1% of the population; 2: 1% - 3%; 3: > 3% of the population.

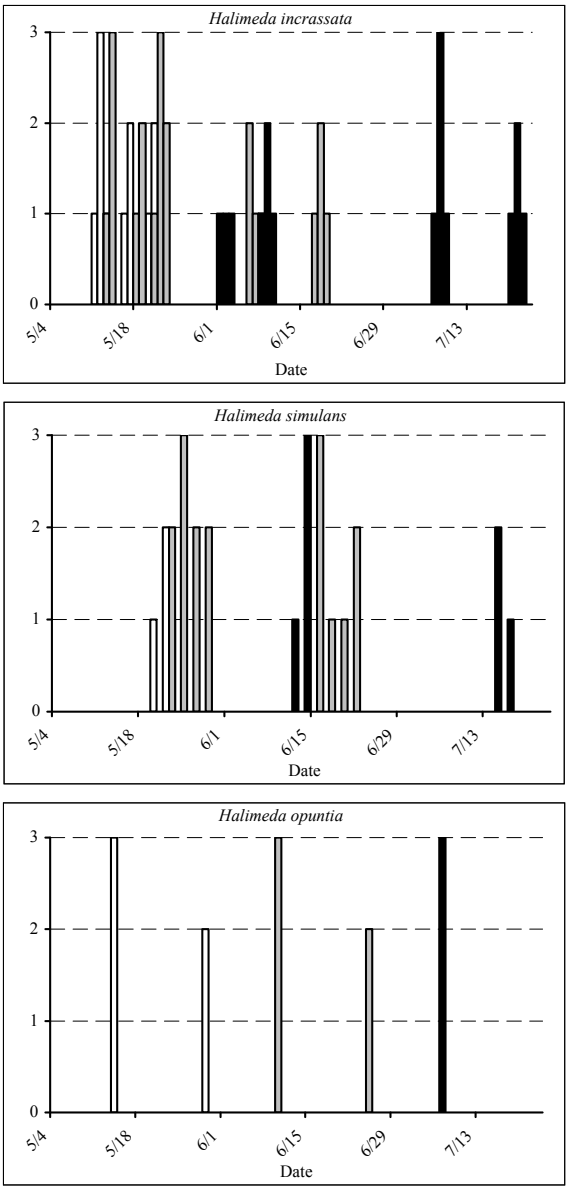

Figure 4: Patterns of sub-seasonal reproduction for three species of *Halimeda* observed during a three-month period in 1999 at three sites in the Caribbean. *H. incrassata* and *H. simulans* are closely related species that occupy similar soft bottom habitats. *H. opuntia* is a larger, sprawling species that occurs on reefs and in soft bottom habitats. Specific dates of observation: Panama: 5/4-6/29/99; St Croix, USVI: 5/4-6/28/99; Florida: 5/20-7/23/99. Shading and axes as in Fig. 3.

Other species showed quite different sub-seasonal patterns of reproductive activity. For example: *Halimeda incrassata* tended to reproduce in three day pulses, separated by gaps of 7 – 14 days; *Halimeda simulans* tended to reproduce every other day at somewhat varied levels of intensity for a several reproductive events, with less regular gaps between these pulses; and *Halimeda opuntia* tended to reproduce roughly every 14 days, with some variance around this tendency (Fig. 4). As with *Caulerpa cupressoides*, however, interspecific differences in the sub-seasonal timing of reproduction by different species of *Halimeda* were essentially preserved at all three sites, despite their differing latitude. Similar trends were found for four species of *Penicillus* (*P. capitatus*, *P. dumetosus, P. lamouroxii*, and *P. pyriformes*) as well as *Udotea flabellum*. *P. capitatus* and *P. lamorouxii* generally showed highly overlapping sub-seasonal patterns of reproduction (i.e., they showed a strong tendency to reproduce on the same day) as did the heterogeneric pair of *P. pyriformes* and *U. flabellum*. This latter pair tended to release gametes 2-3 days following bouts of reproduction by the former pair and, as with other species, this trend was preserved across a latitudinal gradient.

Diel patterns of reproduction

Tropical green seaweeds release their gametes just prior to sunrise and the precise timing of this release is both highly predictable and species-specific (Clifton 1997, Clifton and Clifton 1998). For all species the timing of gamete release, relative to sunrise, was observed to shift to an earlier time with increasing latitude.

For example, the timing of gamete release for *Halimeda tuna* at the three Caribbean sites shifted approximately 30 seconds earlier for every degree of change in latitude across the 15 degree change in latitude across the region (Fig. 5). This trend was further supported by a single observation of gamete release by *H. tuna* from the more northerly, Adriatic Sea. A quadratic curve fitted to the mean time of release at each location ($y = -0.011x^2 - 0.107x - 11.37$) provided an excellent fit to these points (Fig. 5; $R^2 = 1.00$; n = 4; $p = 0.01$).

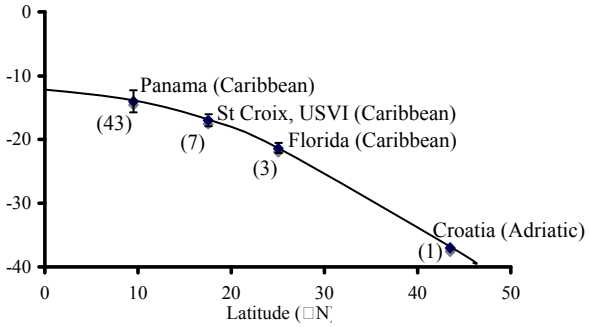

Figure 5: Quadratic curve fit to the mean time of gamete release for *Halimeda tuna*, relative to sunrise and as a function of latitude. Data from Panama are cumulative from observations in 1996-1998. Observations at the other three sites were made in 1998-1999. The single observation of gamete release in the Adriatic (43°9' N, 16°11'E) was provided by Ante Zuljevic. Sample sizes are in parentheses and error bars are ± 2 standard errors.

Discussion

As a general result, diel and seasonal patterns of broadcast spawning by tropical green seaweeds were found to shift with latitude, while sub-seasonal patterns of reproductive behavior were relatively unchanged, irrespective of distance from the equator. These results provide clues as to the proximate mechanisms structuring patterns of temporal synchrony in these broadcast spawning organisms.

Changes in the seasonal peak of reproduction by tropical green seaweeds to a later time of year in higher latitudes follows the trend seen in some stony corals, although simultaneous observations at several sites spanning large latitudinal distances are not common (see Baird et al. 2002; Wilson and Harrison, 2003; Nozawa, et al. 2006). For tropical green algae, the strong correspondence of the onset and cessation of seasonal reproductive activity at higher latitudes with the presence of water temperatures greater than 27°C strongly implicates a role for temperature as an important determinant of the timing of seasonal patterns of reproduction.

Unlike many other broadcast spawning organisms, tropical green seaweeds do not show lunar or tidal patterns of sub-seasonal reproduction (Clifton 1997, Clifton and Clifton 1998), yet the data presented here suggest there is some temporal structure to their patterns of reproduction from day to day. Although these are quite variable from species to species, the relative duration of inter-spawn intervals does show some within species specificity, as well as some intriguing patterns of correlation between species pairs such as *Penicillus capitatus* and *P. lamouroxii* or *P. pyriformes* and *Udotea flabellum*. While the proximate cues that help organize sub-seasonal bouts of reproduction for these seaweeds is not revealed from the results here, the consistency of pattern that was retained, even across a latitudinal gradient of 15° suggests that such organizing cues do exist and the results described here should encourage further study of this topic.

On a diel temporal scale, the shift to an earlier time of gamete release with an increase in latitude supports the idea that changing levels of pre-dawn light are an important component of what structures the exact timing of gamete release on a given morning. The curvilinear aspect of the relationship between time of release and latitude, as shown for *Halimeda tuna* in Fig 5, suggests that the trigger for gamete release may be more a function of cumulative light exposure rather than an absolute threshold of light level.

While much work remains to be done on the proximate mechanisms that organize and promote bouts of gamete release by broadcast spawning organisms, the results presented here suggest that there is utility in the study of gamete release that is done on a variety of temporal and spatial scales. As such, future studies in this arena should be encouraged.

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The effects of temperature and light on the gametogenesis and spawning of four sea urchin and one sea cucumber species on coral reefs in Kenya

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Abstract. Seasonality in reproduction in temperate marine invertebrates is well recognized, but reproduction at the equator where environmental variability is lower is typically assumed to be continuous. This paper reviews studies of the reproductive cycles of four species of sea urchins and one species of sea cucumber to test for seasonality and sexual synchrony along the Kenyan coast (1^0S - 5^0S). Gonad index measurements and macro and microscopic observations of gonads were used to evaluate monthly changes in gonad growth. The urchin *Echinometra mathaei* and sea cucumber *Holothuria arenacava* displayed a seasonal reproductive pattern with gametogenesis commencing in July during the southeast monsoons and spawning peaking between March-April during the northeast monsoons. Monthly temperature and light significantly correlated with gonad index in these species but light displayed a stronger response. The sympatric urchins *Diadema savignyi* and *D. setosum* had a monthly gametogenic cycle with *D. savignyi* spawning after full moon and *D. setosum* spawning in the last quarter. These species also showed seasonal gonad growth with *D. savignyi* following a pattern similar to *E. mathaei.* The life history strategies of these species included a high level of sexual synchrony and spawning at the time most favorable for larval growth.

Key words: Reproductive seasonality, sea cucumbers, sea urchins.

Introduction

Reproduction in echinoids and holothuroids is reported to display seasonal reproductive cycles with temperate species generally having discrete spawning periods and tropical species reproducing for longer periods throughout the year (Orton 1920, Giese and Pearse 1974, Smiley et al. 1991). Reproductive activity was therefore hypothesized to be less synchronized and continuous throughout the year at the equator since environmental variability is less pronounced (Orton 1920, Giese and Pearse 1974, Pearse 1974). However, few studies have compared the reproduction of several species of echinoderms at the same location at the equator (Pearse 1974).

Gametogenesis in echinoderms has been correlated with environmental factors such as water temperature and photoperiod, while spawning is suggested to be triggered by changes in water temperature, food availability, light intensity, water turbulence, salinity and phytoplankton blooms (Krishnaswamy and Krishnan 1967, Himmelman 1980, Engstrom 1980, Cameron and Fankboner 1986, Pearse et al. 1986, Ramofafia et al. 2000, 2003). These factors often vary spatially and temporally and are suggested to exercise a strong selective pressure promoting reproductive synchrony and success.

The Kenyan coast occurs at the equator (1^0S – 5^0S) but is exposed to monsoonal seasonality with varying oceanographic conditions of temperature, light, and productivity (McClanahan 1988). This makes it suitable for testing the effects of monsoonal seasonality on breeding patterns of marine invertebrates at the equator. This paper reviews studies of four species of sea urchins and one species of sea cucumber that were carried out on the Kenyan coast (Muthiga, 2003, 2005, Muthiga and Jaccarini 2005, Muthiga 2006), to test the hypothesis that reproduction will show a weak seasonal pattern at the equator due to reduced variability of the environmental factors that control gametogenesis and spawning.

Materials and methods

The studies were undertaken at Kanamai an unprotected reef and Ras Iwatine within the Mombasa marine reserve located between 3^0S and 5^0S. Study sites are described in Muthiga and Jaccarini (2005), and Muthiga (2005, 2006). At each site ten to twenty specimen were collected each month for a period of one to three

years between 1986 and 1998 (Table 1). The annual reproductive pattern was assessed by tracking gonad growth by measuring the monthly gonad index calculated as the monthly mean ratio of the wet weight of the gonad, relative to the wet weight of the individual for sea urchins (Gonor 1972) and of the wet weight of the body wall for sea cucumbers (Conand 1981). Macroscopic and microscopic observations were also made of the gonad to verify the sex and maturity stages.

Table 1. The locations, species, year of study and SST (mean annual) of the study sites on the Kenyan coast. The mean annual SST is calculated from NOAA data.

Reef	Lat	Species	Year	SST
Kanamai	3° 55'	*T. gratilla*	87-88	26.9
Kanamai	3° 55'	*D. savignyi*	92-93	26.9
Kanamai	3° 55'	*D. setosum*	92-93	26.9
Kanamai	3° 55'	*E. mathaei*	92-94	26.9
Ras Iwatine	4° 70'	*H. arenacava*	97-98	27.1

The relationship between individual size and gonad growth was determined by a correlation between the gonad index (%) and the weight (g) of individuals. The level of synchrony in reproduction between the sexes was determined by a correlation between the mean monthly gonad index of female and male individuals.

Temperature and light measurements were obtained from NOAA climatic data for each location and averaged monthly from 1985 to 1994 and correlated against mean monthly gonad indices for each species. The northeast monsoons start in October and end in April, while the southeast monsoons occur from May to September on the Kenyan coast (McClanahan 1988).

Lunar periodicity was measured in the sea urchins by inducing individuals to spawn using KCl every 2 to 4 days during the peak reproductive period. The gametogenic cycle was also tracked by calculating the mean gonad index every 2 – 4 days for several lunar cycles (new moon = lunar day 0) during the peak period of reproduction.

Results

There was a significant and positive relationship between individual gonad weight and body size for all species ($p < 0.0001$ for all species) and no significant relationship between gonad index and body size for any of the species (Table 2).

The pattern of gonad growth in *E. mathaei* measured through mean monthly gonad indices was seasonal and showed that gametogenesis commenced in July when gonad indices were 3.03 ± 0.54% (sem). The gonad index then gradually increased to peak at 8.03 ± 0.21% between January and February at the peak of the northeast monsoons (Fig. 1). There was no significant difference between the overall average gonad index of females and male *E. mathaei* (Table 3.).

Table 2. The relationship between body size (weight for sea urchins and body wall weight for the sea cucumbers) and the gonad weight, and gonad index of individuals for the species sampled. For each comparison, the correlation coefficient r, and the sample size (in brackets) are provided.

Species	Size vs. gonad weight	Size vs. gonad index
T. gratilla	0.53 (163) *	0.08 ns
D. savignyi	0.60 (270)*	0.14 ns
D. setosum	0.56 (276)*	0.15 ns
E. mathaei	0.41 (100) *	0.03 ns
H. arenacava	0.51 (720)*	0.30 ns

* = $p < 0.0001$; the sample sizes are the same for both comparisons

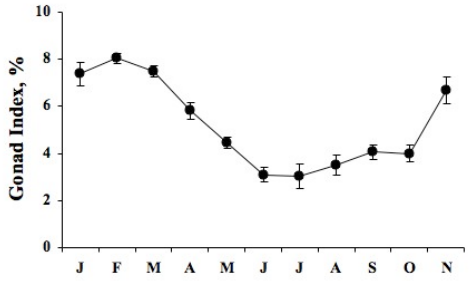

Figure 1. The seasonal variation in the gonad index of *Echinometra mathaei* (± sem) collected at Kanamai (3^0S) between 1992 and 1994.

Monthly spawning induction showed that many individuals of both sexes were ready to spawn by October when 50% of female and 60% of male gonads had ova or sperm filling more than 50% of the gonadal lumen (Fig. 2).

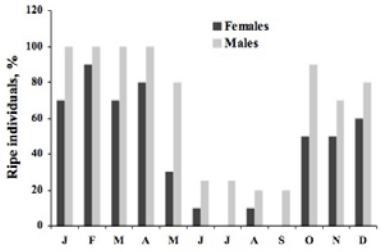

Figure 2: The percent of ripe males and females of *Echinometra mathaei* collected at Kanamai between 1992 and 1994. Ripe individuals were classified as those with gonadal lumen > 50% full of sperm or ova (Pearse 1969).

Histological observations of the gonads showed that the ovarian tubules of *E. mathaei* had in-growing nutritive phagocytes in July, new small oocytes appeared in August and ova were

present from October to May. Males were ripe for ~8 months since sperm filled more than 50% of gonadal lumen from October to May (Fig. 2), while females were ripe for a period of 5 months (December to April).

The pattern of gonad growth was also seasonal in the sea cucumber *Holothuria arenacava* (Fig. 3). Mean monthly gonad indices peaked in March when gonad indices were 13.55 ± 0.45%. The main spawning commenced by August and was completed by May when gonad indices were reduced by more than 90% and all individuals had very small immature gonads.

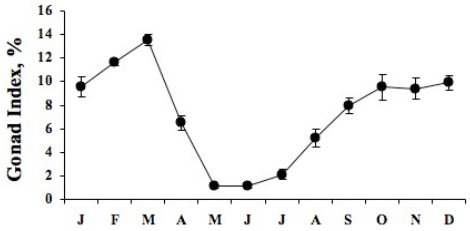

Figure 3: The seasonal variation in the gonad index of *Holothuria arenacava* (± sem) collected at Ras Iwatine (4^{0}S) on the Kenyan coast.

Macroscopic examination of the gonad tubules indicated that gametogenesis commenced in July when small oocytes were present in the gonad tubules. Large oocytes were evident and filling gonad tubules by September.

The sympatric species *Diadema savignyi* and *D. setosum,* showed a monthly gametogenic cycle (Fig. 4.). *Diadema savignyi* had a tightly synchronized gametogenic cycle with ~ 90% of individuals producing copious gametes on lunar day 18 a few days after the full moon.

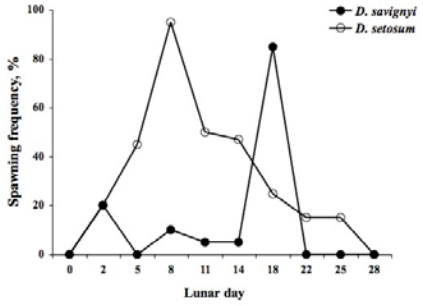

Figure 4: The spawning frequency of *Diadema savignyi* and *D. setosum* induced to spawn with injections of KCl. Spawning induction was carried out every 2 – 4 days for 3 complete lunar cycles. Lunar day 0 = new moon.

Microscopic examination of the gonads showed mature ova in gonadal lumen between lunar day 10 and 17. Spawning in *D. setosum* was less synchronized among individuals and occurred a few days after the new moon for several days (lunar day 5 – 14) with a peak at lunar day 8. Microscopic examination showed ova in individuals from lunar day 0 to lunar day 7.

The annual pattern of gonad growth of *D. savignyi* and *D. setosum* was also seasonal but varied between these sympatric species (Fig. 5).

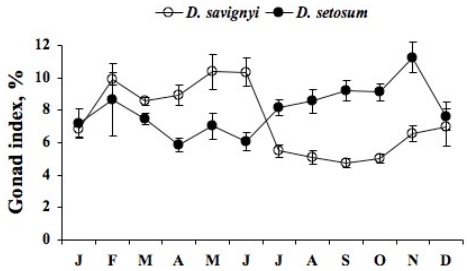

Figure 5: The seasonal variation in the gonad index of *Diadema savignyi* and *D. setosum* (± sem) collected at Kanamai (3^{0}S) on the Kenyan coast.

Although mature gametes were present every month in both species, reproductive output varied and gonad indices were highest between February and June in *D. savignyi* (9.9 – 10.4%) while peak gonad indices occurred between September and November in *D. setosum* (9.2 - 11.3%).

The annual pattern of gonad growth in the sea urchin *Tripneustes gratilla* showed a weak seasonal pattern (Fig. 6) and gonad indices ranged from 2.62 ± 0.23% in January gradually increasing to 4.90 ± 0.64% in July. There was a ~ 30% decrease in gonad index in August.

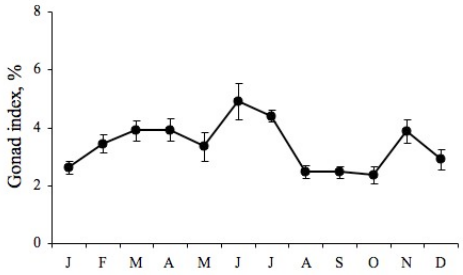

Figure 6: The seasonal variation in the gonad index of *Tripneustes gratilla* (mean monthly ± sem) collected at Kanamai (3^{0}S) on the Kenyan coast.

Macroscopic examination of the gonads revealed that most individuals had 'runny' gonads that could easily be sexed through the presence of female or male gametes. The proportion of individuals within each monthly sample with ripe gonads ranged from 0% to 75% and did not correlate significantly with mean monthly gonad index (r = 0.33).

The gonad indices of *T. gratilla* collected every few days over a period of two lunar cycles from April to July indicated lunar periodicity. Gonad indices increased from 3% on lunar day 0 (new moon) to 8% by lunar day 8 and lowered to < 2% between lunar days 18 to 25 during the last quarter of the moon.

The relationship between the reproductive activity of males and females measured as the average monthly gonad index in *E. mathaei, T. gratilla* and *H. arenacava* was highly significant but showed a lower level of synchrony in *D. savignyi* and *D. setosum* (Table 3.)

Table 3. The average gonad index of female and male individuals and the relationship between mean monthly gonad index of females and of males of the studied species. For the monthly comparison between sexes, the correlation coefficient r are provided.

Species	Females (GI %)	Males (GI%)	F vs. M r
T. gratilla	5.46±0.42 (55)	5.47±0.54 (47)	0.91** (12)
D. savignyi	8.03±0.35 (36)	7.03±0.37 (37)	0.92 * (12)
D. setosum	9.39±0.6 (31)	8.86±0.56 (28)	0.80 * (12)
E. mathaei	6.94±0.22 (246)	7.16±0.21 (205)	0.75 ** (12)
H. arenacava	9.8±0.3 (240)	6.8±0.4 (196)	0.79 ** (12)

* = $p < 0.05$ level; ** = $p < 0.01$; samples sizes in brackets

The relationship between temperature and light was variable between species. *Echinometra* mathaei and *H. arenacava* showed significant relationships with both temperature and light but the relationship with light showing the stronger response in both species (Table 4).

Table 4. The relationship between mean monthly gonad index of each species and mean monthly SST (°C), and mean monthly light (Einstein/m^2/day) at the studied sites. For each comparison, the correlation coefficient r, and the p value (in brackets) are provided.

Species	Gonad index vs. temperature	Gonad index vs. Light
T. gratilla	-0.20 (0.52) ns	-0.43 (0.16) ns
D. savignyi	0.64 (0.02) ns	- 0.19 (0.55) ns
D. setosum	- 0.39 (0.20) ns	0.39 (0.2) ns
E. mathaei	0.69 (< 0.01)	0.75 (< 0.01)
H. arenacava	0.71 (0.01)	0.94 (< 0.0001)

There was no significant relationship between the monthly gonad index and temperature and light in *D. savignyi*, *D. setosum* and *T. gratilla*. The highest gonad indices in both *D. savignyi* and *D. setosum* however coincided with the months with the highest temperatures and light.

Discussion

The gonad index was variable across all sizes and did not show a correlation with body size in the species studied. This indicates that gonad index is independent of body size in these species and is a reliable predictor of reproductive condition as has been shown for other species of sea urchins (Gonor 1972, Muthiga 1996) and sea cucumbers (Drumm and Loneragan 2005).

The pattern of gonad growth was seasonal in all the species with *E. mathaei* and *H. arenacava* showing a particularly strong pattern that included complete spawning and resorption of gonads in May-June during the southeast monsoons followed by a period of gonad growth that peaked between February and March during the northeast monsoons.

This pattern is comparable to marine invertebrate species that occur at higher latitudes (Smiley et al. 1991, Ramofafia 2000, Drumm and Loneragan 2005) and does not support the hypothesis that marine organisms at the equator will breed year round (Orton 1920; Giese and Pearse 1974; Smiley et al. 1991). Previous studies in the tropics but at latitudes higher than the equator have also shown seasonal patterns of reproduction in sea urchins and sea cucumbers (Pearse 1969, 1974, Reichenbach 1999, Ramofafia et al. 2000, 2003).

The pattern of gonad growth in *D. savignyi*, *D. setosum* and *T. gratilla* also showed an annual pattern but in all three species, the presence of gonads and gametes in individuals during most months of the year indicated that individuals were reaching reproductive maturity and spawning at different times of the year. Gonad growth however was higher during the northeast monsoons for *D. savignyi* and *T. gratilla* and started earlier during the southeast monsoons in *D. setosum* and peaked at the beginning of the northeast monsoons.

Temperature and light, the main environmental factors that have been shown to trigger gametogenesis and spawning in marine invertebrates showed relatively small variations on the Kenyan coast. However, in at least two of the studied species, gonad growth correlated significantly with light and temperature suggesting that these factors played a role in controlling reproduction in these species.

The presence of reproductive periodicity functions to allow individuals of a population to take advantage of the best time of the year to produce young and synchronize spawning (Lessios 1981). In the studied species male and females were highly synchronized and spawning

activity generally peaked at or just prior to the time when phytoplankton concentrations peaked on the Kenyan coast.

Although the monthly ranges of temperature and light at the studied sites were low (± ~4^{0}C; ~20 Einstein/m^2/day), the changes were predictable and followed a strong seasonal pattern that is driven by the monsoons (McClanahan 1988). This predictability could act as a reliable cue for the onset of gametogenesis and spawning.

The fact that the sympatric *Diadema* species – that also showed a monthly spawning pattern, displayed a seasonal pattern of gonad growth and the presence of seasonal reproduction in fish (Ntiba and Jaccarini 1990), in sea cucumbers (Kithakeni and Ndaro 2002, Muthiga per comm.) and recruitment of barnacles and oysters (Ruwa and Polk 1994) suggests that monsoonal seasonality maybe the proximate driver of life history strategies of marine invertebrates on the east African coast.

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Survival of Brooding and Broadcasting Reef Corals Following Large Scale Disturbances: Is There Any Hope for Broadcasting Species During Global Warming?

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Abstract. The reproductive ecology of extant equatorial eastern Pacific (EEP) zooxanthellate scleractinian corals is examined relative to projected global warming conditions. Life history characteristics and patterns of abundance and distribution of 13 recently studied species in Costa Rica, Panamá, and the Galápagos Islands are considered. Twelve of these are broadcast spawners, which are numerically abundant, widely distributed, and the principal EEP reef builders. *Porites panamensis*, the lone brooder, is a small nodular coral with a restricted distribution. The majority of the broadcasting species are highly fecund, release gametes during large parts of the year, and produce sexual recruits that appear to disperse relatively far to diverse habitats. Asexual fragmentation is also common in most EEP broadcasting species compared to *P. panamensis*. We hypothesize that diverse habitats and varying environmental conditions (e.g., upwelling/nonupwelling centers, varying thermal conditions, high/low turbidity, high/low productivity, El Niño/La Niña activity) increase the probability of survival of sexual recruits and the asexual fragments of broadcasting species. Surviving refuge populations could serve as sources to repopulate degraded coral communities. The EEP reef coral fauna, dominantly broadcasting species, possess traits (e.g., high fecundity, widely dispersed) that could help resist environmental challenges during global warming.

Key words: Broadcast-spawning coral survival, eastern Pacific.

Introduction

During multiple and diverse disturbances to coral reefs since the early 1980s, the perception has gained favor that corals brooding and releasing planula larvae have higher survival rates and subsequent success in reproduction and recruitment to degraded reefs than broadcast-spawning corals. This view was especially supported by studies in the Caribbean (e.g., Aronson and Precht 2001; Knowlton 2001). Following hurricanes, coral disease outbreaks, bleaching events and other disturbances, it was found that brooding species on many reefs demonstrated higher rates of survivorship and recruitment than broadcasting species. Relatively small colonies of brooding species have replaced major frame-building species on several western Atlantic coral reefs.

The cool/turbid and elevated nutrient conditions associated with upwelling in early Miocene time was postulated to have led to the demise of about one-half of the coral genera in the Caribbean. From an analysis of the coral genera that survived these changing conditions during the Caribbean Oligocene-Miocene extinction event, Edinger and Risk (1995) concluded that brooding corals were disproportionately favored over broadcasting coral taxa. Edinger and Risk (1995) extended their analysis to the modern eastern Pacific, suggesting that the upwelling environments in the equatorial eastern Pacific (EEP) would also favor the survival of brooding corals. At the time of Edinger and Risk's (1995) study, little was known of the reproductive modes of eastern Pacific corals. Several studies are now available, providing information for a re-examination of the relative benefits of brooding and broadcasting reproductive modes in marginal reef settings. Here we examine these contrasting modes of reproduction in the EEP region, in the context of coral bleaching caused by elevated temperature stress.

Material and Methods

This re-analysis is based on the reproductive biology and ecology of EEP zooxanthellate scleractinian corals, chiefly from study sites in Costa Rica, Panamá and the Galápagos Islands (Fig. 1). Life history information was compiled from Glynn et al (1991 1994 1996 2000); Colley et al (2002 2006); and unpublished observations. The 13 species considered, of the 23 known species constituting the EEP fauna, are the principal reef-building corals. This study examines physical environmental conditions,

reproductive traits, spatial/seasonal reproductive patterns, and geographic distributions from the published literature and unpublished sources.

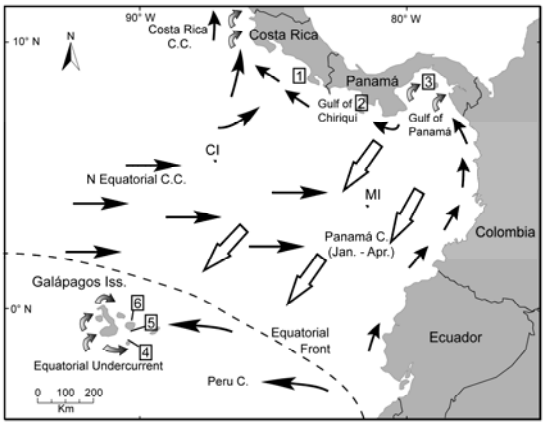

Figure 1: Coastal and offshore island sites of the equatorial eastern Pacific region. Numbered study sites: 1 – Caño Is, Costa Rica; 2 – Uva Is, Panamá; 3 – Saboga Is, Panamá; 4 – Floreana Is, 5 – Santa Cruz Is, 6 – Canal de Itabaca, Galápagos Islands. Locations of surface currents and upwelling centers (small thick arrows off NW Costa Rica, in Gulf of Panamá and W sector of Galápagos Iss) are noted. Key: Costa Rica C.C., Costa Rican Coastal Current; N Equatorial C.C., North Equatorial Counter Current; Panamá C., seasonal Panamá Current (large open arrows); Peru C., Peru Current; CI, Cocos Island; MI, Malpelo Island.

Results

Of the 13 studied coral species, 12 spawn gametes (Table 1). These include the major frame-building species *Porites lobata*, *Pavona gigantea*, *Pavona clavus* and *Gardineroseris planulata*, which construct massive (dome-like) colonies, and *Pocillopora damicornis* and *Pocillopora elegans* that form branching colonies. Massive colonies attain 1 m in diameter and occasionally 2-3 m. Branching colonies range from 15 to 30 cm in diameter, and when growing in juxtaposition, form vertically-oriented and interlocking branches ≥ 1 m in height. Although no data exist for *Pocillopora inflata* from the majority of study sites, they spawned gametes immediately after collection in the Pearl Islands, Panamá (pers obs). *Porites panamensis* is the only brooding species. It typically forms small (5-10 cm max diam) nodular colonies, and occasionally colonies up to 30 cm in diameter that encrust basalt substrates.

Five broadcast-spawning species and the brooder *Porites panamensis* are gonochoric. Six broadcast spawning species are hermaphroditic: two pocilloporids are simultaneous hermaphrodites and four agariciids are sequential cosexual hermaphrodites. Sequential cosexual hermaphrodites are those species that display multiple cycles of gamete development during a breeding season; maturation of the sexes alternates in tissues, revealing early stage gametes of one sex with late stage gametes of the opposite sex (Policansky 1982). Asexual fragmentation occurs occasionally to commonly in all species except *Porites panamensis* and *Psammocora superficialis*.

Table 1. Life history traits of presently studied EEP zooxanthellate coral species. SG-spawns gametes, BP-broods planulae; sexuality: G-gonochoric, H-hermaphroditic, simul-simultaneous, seq, sequential cosexual; Growth form (greatest dimension): nod-nodular, mas-massive, branch-branching, encrust-encrusting, pet-petaloid, sm-small (5-10cm), med-medium (10-30cm), lg-large (0.3-2m); Asexual reproduction: rare-seldom observed, occ-occasional, sometimes observed, com-common, frequently observed. Environmental conditions: Gulf of Panamá, seasonal upwelling; Gulf of Chiriquí, nonupwelling, seasonal shoaling of thermocline; Galápagos Islands, seasonally cool. Numbers in bold indicate number of months during the calendar year when Stage IV oocytes were present in histological sections. Numbers in parentheses indicate number of non-overlapping months when Stages I-III oocytes were present. n/a, species not present, too uncommon or rare to sample, or not sampled seasonally.

Species	Sexuality	Growth form	Asexual reprod.	No. of months with mature ovaries		
				Gulf of Panamá	Gulf of Chiriqui	Galápagos Islands
Porites panamensis (BP)	G	nod, encrust, sm	Rare	**8** (2)	**12**	n/a
Porites lobata (SG)	G	mas, lg	Com	**0** (1)	**5** (3)	**1** (5)
Pocillopora damicornis (SG)	H, simul	branch, md	Com	**2** (0)	**6** (4)	**1** (0)
Pocillopora elegans (SG)	H, simul	branch, md	Com	**2** (0)	**11** (0)	**3** (7)
Pocillopora inflata (SG)	?	branch, md	Com	n/a	n/a	n/a
Pavona clavus (SG)	G	mas, lg	Occ	**4** (1)	**3** (5)	**6** (2)
Pavona varians (SG)	H, seq	encrust, md	Occ	**4** (2)	**5** (6)	**4** (7)
Pavona gigantea (SG)	H, seq	mas, lg	Occ	**3** (4)	**6** (4)	**7** (5)
Pavona chiriquiensis (SG)	H, seq	encrust, md	Occ	n/a	**5** (6)	**4** (7)
Gardineroseris planulata (SG)	H, seq	mas, lg	Com	**0** (0)	**6** (1)	n/a
Psammocora stellata (SG)	G	branch, sm	Com	**1** (3)	**8** (1)	**6** (2)
Psammocora superficialis (SG)	G	encrust, sm	Rare	n/a	**4** (0)	**3** (2)
Diaseris distorta (SG)	G	petal, sm	Com	n/a	n/a	**4**

The temporal presence of mature ovaries in broadcast-spawners was highly variable, depending on locality, which in turn was strongly influenced by seasonal thermal conditions. In general, species in the thermally stable Gulf of Chiriquí were reproductively active from 3 to 11 months of the year. Six of the 10

broadcast-spawners in the Galápagos Islands contained mature oocytes from 4 to 7 months of the year. Zooxanthellae were present in both the oocytes of *Porites panamensis* and three broadcast-spawning species, namely *Porites lobata, Pocillopora damicornis* and *Pocillopora elegans*. In the seasonally upwelling (late December-through April) Gulf of Panamá, planulae were present in *P. panamensis* during 8 nonupwelling months, and year round in the Gulf of Chiriquí where temperature conditions were high and more stable. Where quantified, fecundity estimates were high for six broadcast-spawning species: *Porites lobata, Pavona clavus* (unpub data), *Pavona varians*, *Pavona gigantea*, *Pavona chiriquiensis* and *Gardineroseris planulata*, and the brooder *Porites panamensis*.

Porites panamensis has the narrowest distribution of all 13 species. Within the eastern Pacific region it is known only from mainland localities and the Revilla Gigedo Islands, Mexico (Table 2). The majority of the broadcast-spawning species are present at several eastern Pacific localities, including all major oceanic island sites (Garzón-Ferreira and Pinzón-C 1999; Glynn and Ault 2000; Guzmán and Cortés 2007). Eleven of the 12 EEP broadcast-spawners are also present in the Indo-Pacific region. *Pocillopora inflata* occurs in the Phoenix Islands (D Obura, pers comm) and *Pavona chiriquiensis* at Wallace Island (M Pichon, pers comm). While P. damicornis occurs from the eastern Pacific across the tropical Indo-Pacific, it is a broadcast-spawner in the eastern Pacific and releases brooded planulae throughout the remainder of its range (Richmond and Hunter 1990).

A global survey of the number of coral taxa that experienced major bleaching disturbances during the past few decades shows relatively high survival and recovery of broadcast-spawning species (Table 3, Baker et al in press). High proportions of the dominant recovering corals in the Indian Ocean, eastern Pacific, central W/S Pacific, and Arabian Gulf were broadcast spawning species. Even in the western Atlantic reductions in the relative abundances of broadcast-spawners were markedly less than brooding species after the 2005 coral bleaching/disease event in the U.S. Virgin Islands (Rogers et al 2008). Agariciid brooders showed the greatest declines in abundance.

Table 2. Known geographic distributions of EEP zooxanthellate corals. BP, broods planulae; SG, spawns gametes. Localities: EPM, eastern Pacific mainland; REV, Revilla Gigedo Islands; CLP, Clipperton Island, ca 1,200 km off Mexican coast; COC, Cocos Island, Costa Rica; MAL, Malpelo Island, Colombia; GAL, Galápagos Islands, Ecuador; CPC, central Pacific; WPC, western Pacific; IOC, Indian Ocean. Double lines for *P. damicornis* denote planula brooding at all studied Indo-Pacific regions.

Species	Eastern Pacific localities						Indo-Pacific regions		
	EPM	REV	CLP	COC	MAL	GAL	CPC	WPC	IOC
Porites panamensis (BP)	----------								
Porites lobata (SG)	----------	----------	----------	----------	----------	----------	----------	----------	----------
Pocillopora damicornis (SG, BP)	----------			-----		-----	==========	==========	==========
Pocillopora elegans (SG)	----------			----------	----------	----------	----------	----------	----------
Pocillopora inflata (SG)	-----			-----		----------			
Pavona clavus (SG)	----------			----------	----------	----------	----------	----------	----------
Pavona varians (SG)	----------	----------	----------	----------	----------	----------	----------	----------	----------
Pavona gigantea (SG)	----------	----------	----------	----------	----------				
Pavona chiriquiensis (SG)	-----			----------				------	
Gardineroseris planulata (SG)	-----			----------	----------	----------	----------	----------	----------
Psammocora stellata (SG)	----------	----------	----------		----------	----------	----------	----------	----------
Psammocora superficialis (SG)	-----			-----		----------	----------	----------	----------
Diaseris distorta (SG)	-----			-----		----------	----------	----------	----------

Table 3. Reproductive mode of predominant recovering coral taxa following major bleaching events worldwide.

Region	Reproductive mode (no)		Predominant recovering taxa
	broadcasters	brooders	
Indian Ocean	11	1	*Montipora circumvallata, Pavona* spp., *Acropora* spp., *Porites* spp., *Galaxea fascicularis*, *Pocillopora* spp., *Fungia* spp.
West Atlantic	4	1	*Montastraea annularis* complex (3 spp.), *Siderastrea siderea*, *Porites porites*
Eastern Pacific	8	0	*Porites lobata*, *Pocillopora* spp., *Pavona* spp., *Psammocora* spp., *Gardineroseris planulata*
Central W/S Pacific	10	2	*Pocillopora damicornis*, *Acropora* spp., *Porites* spp., *Montipora* spp., Faviidae, *Pavona* spp., *Galaxea fascicularis*
Arabian Gulf	7	0	*Porites* spp., *Acropora downingi*, *Platygyra daedalea*, *Leptastrea transversa*, *Favia* spp.

Discussion

Present information on the reproductive traits of EEP reef-building corals indicates that the great majority, 12 of 13 studied species, are broadcast-spawners. Szmant (1986) hypothesized that the survival and recruitment of Caribbean brooding species should be favored in habitats that are subject to frequent disturbances. Such species are typically small, short-lived, reach reproductive maturity at an early age, iteroparous (with 9-12 reproductive cycles yr-1), produce lecithotrophic larvae, host zooxanthellae, and undergo local settlement/recruitment. Some observations have purported to show that the planulae of brooders can potentially disperse over long distances, thanks to the presence of energy-rich food stores and phototrophic zooxanthellae (e.g., Richmond 1987). Even some brooders with zooxanthellate larvae, however, have been shown to have short dispersal ranges with the majority recruiting to within 100 m of their natal colony (Underwood et al 2007). The literature review of Harrison and Wallace (1990) indicates a long pelagic phase for the larvae of broadcast spawning corals compared with brooded planulae. In addition, a recent study quantifying the survival of larvae of broadcast spawners suggests a greater potential for long-distance dispersal than previously recognized (Graham et al 2008).

Some recent studies in the western Atlantic appear to support Szmant's (1986) prediction. For example, brooding poritid and agariciid species have replaced broadcasting acroporid and faviid species on reefs in Jamaica (Hughes 1994) and Belize (Aronson and Precht 2001). In Brazil, small brooding recruits of siderastreid, agariciid and faviid species were much more abundant after unknown disturbances (possibly storm waves and mechanical damage by divers) than broadcast-spawning mussid corals (Kikuchi et al 2003). However, a survey of recovering corals from a bleaching event in the US Virgin Islands revealed a higher survivorship of broadcasting over brooding species (Rogers et al 2008). In this study, it was found that broadcast-spawning species in the *Montastraea annularis* complex remained community dominants whereas brooding *Agaricia agaricites* declined dramatically in abundance.

Edinger and Risk (1995) inferred that the majority of coral genera that survived increased upwelling in the early Miocene in the Caribbean were brooding species, compared with broadcast-spawning species that suffered high rates of extinction. They assumed that the surviving genera of brooding and broadcasting corals followed the same developmental patterns observed in present-day taxa. It is necessary to exercise caution when inferring the reproductive mode of coral species within particular genera. For example, the genera *Porites*, *Acropora* and *Pocillopora* contain species that exhibit brooding and broadcast spawning. *Porites* and *Acropora* were classified as taxa exhibiting a mixed mode of reproduction. In the Caribbean, all *Porites* spp. are brooders, but in the Indo-Pacific most *Porites* spp. are broadcast spawners. Even within the morphospecies *Pocillopora damicornis*, broadcast spawning occurs in the EEP and planula release in the Indo-Pacific (Richmond 1990). Ongoing studies have revealed that the major reef-building corals in the EEP are broadcast spawners and not brooding species as suggested by Edinger and Risk (1995).

During both the 1982-83 and 1997-98 ENSO bleaching events in Costa Rica (Guzmán et al., 1987) and Panamá (Glynn, 1990; Glynn et al 2001) whole colony mortality was high in *Porites panamensis* compared with partial colony mortality in several large broadcast spawning species. *Porites panamensis* demonstrated a delayed bleaching/mortality response, 2-3 months after most other scleractinian species, and disappeared from several monitored sites as well as two entire coral reefs for 3 to 5 years (Glynn 1984). This brooding species was considered regionally endangered by Glynn et al (2001).

An important issue relating to the survival of reef corals of divergent reproductive modes is the discrepancy in time and space on evolutionary and ecological scales. In this analysis, we have considered the survival of particular reproductive groups over evolutionary time. The ecological data supporting these survival patterns, i.e. which groups recover following disturbances over periods of years to a few decades, are at variance with the long-term trends. In some cases, brooders seem to demonstrate higher survival (e.g. Jamaica, Belize, Brazil), supporting Szmant's (1986) predictions, while in other cases broadcast spawning species appear to be more successful at surviving disturbances (e.g. Virgin Islands, Indian Ocean, eastern Pacific). This difference may be due in part to disturbance type (chronic vs acute), site specificity or previous history (Hughes 1989). Over evolutionary time, however, the variability of responses may become reduced with the life history traits of some taxa promoting long-term survival.

Considering the localized settlement behavior of *Porites panamensis* and the widespread distribution of EEP broadcast spawners, we conclude that EEP species with the latter reproductive mode have a high potential for dispersal. In addition, the EEP offers a wide variety of environmental settings for colonization: upwelling/nonupwelling centers, variable temperature regimes, carbonate/basalt substrates, wet/dry season conditions, high/low

turbidity, high/low productivity, and El Niño/La Niña activity. Environmental circumstances that could enhance coral survival during stressful warming events are upwelling centers, shallow thermocline development, and high tidal amplitude with attendant strong current flow (Riegl and Piller 2003). The variety of major surface currents should also promote a relatively high degree of connectivity between EEP localities. In conclusion, the life history traits of broadcast spawning corals, in combination with an opportunity to colonize diverse EEP environments, would appear to enhance the survival of broadcast-spawning corals following large-scale disturbances in this region.

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Multi-specific coral spawning in spring and autumn in far north-western Australia

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Abstract. In Australia, 'mass' coral spawning, where hundreds of coral colonies and numerous species release gametes on the same night, typically occurs in October/November (spring) on the Great Barrier Reef, and in March/April (autumn) on the Western Australian coast. To assess if Western Australian corals also spawn in spring, surveys of the reproductive status of *Acropora* were conducted at over 10 sites in two locations in north-western Australia, spanning over 1000 km and six degrees of latitude. This research found that 39% of *Acropora* species spawned in spring at the most northerly location and 7% of *Acropora* species spawned in spring at the more southerly location. This is the first time such a significant reproductive event in spring has been recorded in spring in Western Australia. While the causal mechanisms driving this second spawning period are likely to be complex, suitable environmental conditions in both spring and autumn might allow for two reproductive seasons on the far north-western Australian coast. This study suggests that the conditions that are suitable for reproduction in October/November are more favorable at the Bonaparte Archipelago than further south at the Dampier Archipelago.

Key words: biannual spawning, coral reefs, spawning synchrony, north-west Australia

Introduction

Multi-specific 'mass' coral spawning, where hundreds of zooxanthellate scleractinian corals synchronously release gametes on the same night (Harrison et al. 1984), has been found to occur on many coral reefs around the world; however, the timing and synchrony of spawning varies considerably among different geographic locations and regions (Guest et al. 2005a).

In some regions around the world, synchronous multi-specific spawning is concentrated over 2-3 months of the year (e.g. Japan, Hayashibara et al. 1993; Central Pacific, Kenyon 2008), while in other regions the length of the spawning period can extend for 5-7 months (e.g. Palau, Penland et al. 2004; Kenya, Mangubhai & Harrison 2008).

In Australia, the traditional paradigm has been that synchronous mass spawning largely occurs annually over 1-2 months, in spring (October and/or November) on the Great Barrier Reef (GBR) (Willis et al. 1985; Babcock et al. 1986), and in autumn (March and/or April) on the Western Australian coast (Simpson 1991; Babcock et al. 1994), and that most colonies of many species spawn over a few consecutive nights.

While corals on many reefs around Australia do display remarkable synchrony in their reproductive timing, there are also some noteworthy exceptions. On the GBR, a number of *Acropora* species are known to spawn several months after the mass spawning period (Wallace 1985; Kojis and Quinn 1981; Wolstenholme 2004), while others may spawn twice a year (Stobart et al. 1992). There is also evidence that in the *Acropora*, some spawning occurs following *every* full moon between October and February on the central GBR (Baird 2006).

Similarly on Western Australian reefs, synchronous spawning of multiple species has been recorded up to two months either side of the mass spawning period (Stewart 1993; Smith 1993; Rosser 2005), and a number of *Acropora* species have also been found spawning in spring (Rosser and Gilmour 2008).

Several environmental factors have been cited as influencing the timing of mass spawning in different regions. The most common include sea temperature, for its assumed role in the growth and maturation of gametes and larvae (Giese and Pearse 1974; Willis et al. 1985; Oliver et al. 1988); rainfall, for its reduction in salinity and physical impact upon gametes (Harrison et al. 1984; Mendes and Woodley 2002); tides and/or calm sea conditions, for their role in promoting fertilization and/or dispersal (Babcock et al. 1986; Hayashibara et al. 1993); and solar insolation, for its role in synchronising gametogenesis (Penland et al. 2004; van Woesik et al. 2006).

The extent to which corals participate in synchronous multi-specific spawning outside the traditional 'mass spawning period' was the focus of this research. We investigated the proportion of *Acropora* species that were likely to spawn in spring (October/November) at two locations in north-western Australia.

Material and Methods

This research was conducted at the Dampier Archipelago (20º28'S, 116º37'E) and the Bonaparte Archipelago (14º30'S, 125º0'E) (Fig. 1) in north-western Australia. Expeditions to each location were conducted independently by the authors (Rosser, Bonaparte; Baird, Dampier) as part of larger projects.

Reproductive samples were collected between 18 and 20 March at Bonaparte, and between 26 and 28 March at Dampier (full moon 2 April 2007); and between 9 and 10 October at Dampier, and between 23 and 25 October at Bonaparte (full moon 26 October 2007). Coral spawning typically occurs 8-10 days after the full moon in Western Australia so it was anticipated that spawning would occur in April and November.

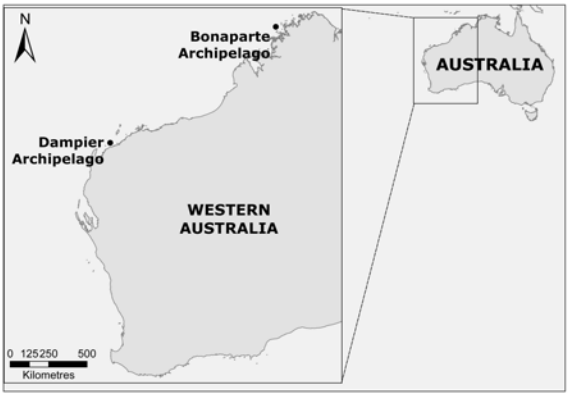

Figure 1: Location of the study sites in Western Australia

Surveys of the reproductive status of corals from the genus *Acropora* were conducted at both locations. Reproductive status was assessed by breaking up to three branches of each colony of approximately 30 species of *Acropora* (up to 700 colonies were sampled at each location). Colonies were assessed *in-situ* and scored for the presence of pigmented eggs prior to the full moon. This sampling methodology has been used previously by Harrison et al. (1984) and Baird et al. (2002) as pigmented eggs represent mature eggs that will be released shortly after the next full moon.

A lower number of *Acropora* species were sampled at the Dampier Archipelago (14 species) than at the Bonaparte Archipelago (31 species) in October due to the species assemblages in each location being different; the Bonaparte Archipelago has a much higher species diversity than the Dampier Archipelago.

Geophysical parameters including mean monthly records of sea surface temperature (SST), wind speed and rainfall for 2007 (sourced from Remote Sensing Systems satellites sponsored by NASA http://www.remss.com/) were also compared between the two locations.

Results

In the Bonaparte Archipelago, 12 species of *Acropora* (39% of the *Acropora* species sampled) were recorded with pigmented eggs in October 2007, indicating that they were likely to spawn in November (Fig. 2). Dampier Archipelago however showed a different result, with only one *Acropora* species (7% of *Acropora* species sampled) recorded with pigmented eggs in October (Fig. 2).

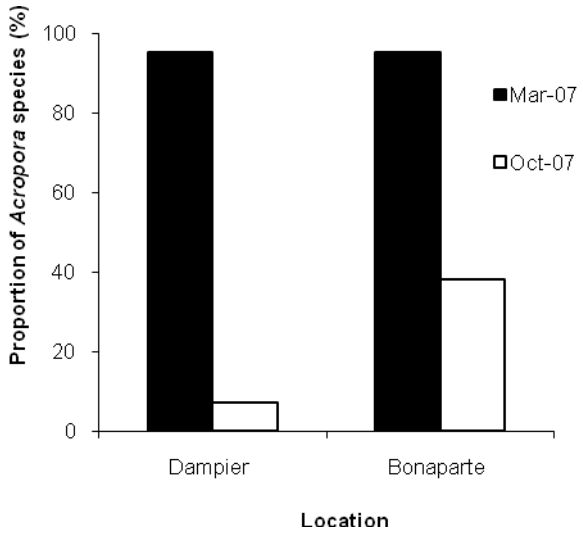

Figure 2: The proportion of *Acropora* species with pigmented eggs in March and October 2007 at the two study locations.

Ten species were sampled at both sites; of these, four species had mature colonies (40%) at Bonaparte, while only one species *Acropora humilis* (10%) had mature colonies at Dampier. For one of the four mature species at Bonaparte (*A. latistella*), only two colonies were sampled at Dampier (Table 1). For the other two mature species at Bonaparte, *A. digitifera* and *A. loripes*, 35 and 11 colonies were sampled respectively at Dampier but no colonies were found containing mature oocytes. At Bonaparte, 8 other species had colonies with mature oocytes, but these species were not sampled at Dampier (Table 1).

Table 1: The proportion of colonies (%) with pigmented eggs in each species in October 2007 from the two study sites.

	Dampier		Bonaparte	
	n	% pigm	n	% pigm
Acropora anthocercis			1	100
Acropora aspera			11	0
Acropora austera			4	0
Acropora bifurcata	3	0		
Acropora cerealis			2	0
Acropora clathrata			7	0
Acropora cytherea			1	100
Acropora dendrum			2	0
Acropora digitifera	35	0	14	36
Acropora divaricata	4	0		
Acropora echinata	3	0		
Acropora florida			16	0
Acropora gemmifera			14	0
Acropora humilis	13	62	34	82
Acropora hyacinthus			27	7
Acropora intermedia			20	0
Acropora latistella	2	0	4	100
Acropora loripes	11	0	16	6
Acropora microclados			1	0
Acropora microphthalma			2	0
Acropora millepora	36	0	18	0
Acropora muricata	2	0	13	0
Acropora nasuta	35	0	8	0
Acropora papillare			2	0
Acropora pulchra			2	0
Acropora pallida			1	100
Acropora robusta	1	0	4	0
Acropora samoensis			35	43
Acropora sarmentosa			19	16
Acropora secale			6	33
Acropora selago	23	0		
Acropora spicifera	5	0	2	0
Acropora striata			1	0
Acropora tenuis	6	0	13	0
Acropora valida			19	63

The proportion of colonies within each species that had pigmented eggs in October at Bonaparte Archipelago was highly variable, ranging from 5-100% (Table 1), with a mean of 47% ±7.6 (SE).

Interestingly, at both Dampier and Bonaparte Archipelagos, 95% of *Acropora* species had pigmented eggs in March 2007 (Fig. 2).

A comparison of geophysical parameters across the two locations showed that SST was higher at the Bonaparte Archipelago than at the Dampier Archipelago, though the annual variation in SST was similar at both locations (4ºC).

Average monthly wind speed showed that in the Bonaparte Archipelago there were two distinct periods of low winds: March and October, while in the Dampier Archipelago wind speed was more constant, with a drop in wind speed occurring only in March.

Monthly rainfall patterns were similar at both locations, with the highest monthly rainfall occurring in March (at both locations).

Discussion

Here we provide clear evidence that at the Bonaparte Archipelago in Western Australia, multi-specific spawning is occurring twice a year, in both spring and autumn, with March/April being the primary spawning period and October/November being the secondary spawning period.

Our finding that a substantial proportion of the *Acropora* populations in north-western Australia participate in a second mass spawning event is of particular interest because previously, the secondary spawning event was considered to be relatively minor. Rosser and Gilmour (2008) found that 36% of *Acropora* species were likely to spawn between October and December at Barrow Island (in north Western Australia), and the mean proportion of colonies in each species participating was 27% ± 7.4 (SE). The spring spawning event at Bonaparte Archipelago described here, involves a greater number of *Acropora* species and almost double the proportion of colonies from each species participating.

Multi-specific spawning periods in both spring and autumn have been recorded in other regions, however the number of species and colonies involved is generally much lower (Wolstenholme 2004; Guest et al. 2005b).

We provide no evidence to suggest that individual colonies spawned twice, because most *Acropora* species are considered to have a single annual gametogenic cycle (Wallace 1985; Mangubhai and Harrison 2008; Rosser & Gilmour 2008). Rather, we suggest that different individuals within a single species spawn at different times.

The most northerly location, Bonaparte Archipelago, had a higher number of *Acropora* species spawning in November 2007 than at the southern location in the Dampier Archipelago. While a greater number of species was sampled at Bonaparte than at Dampier due to different species assemblages at each location, the trend in this data suggests that the magnitude of spring spawning is greater at

Bonaparte than at Dampier. The causal mechanisms resulting in differential spawning patterns are likely to be complex and have yet to be fully resolved. The comparison of geophysical data between the two locations may, however, provide some insight into causal mechanisms.

In October and November, sea surface temperature was 2-3ºC lower at the Dampier Archipelago than at the Bonaparte Archipelago, and this may explain why the magnitude of spawning was much lower at Dampier in November. A meta-analysis of coral spawning in the Caribbean showed that monthly mean SST has a significant relationship with gamete release, therefore suggesting that an optimal temperature is necessary for gamete maturation and that this temperature window may be quite narrow (van Woesik et al. 2006). In this respect, it is possible that sea surface temperature is sub- optimal for gamete maturation in October/November at the Dampier Archipelago.

Secondly, there was a distinct drop in mean monthly wind speed during October and November at the northerly location, whereas wind speed remained relatively constant throughout spring at the Dampier Archipelago (a drop in wind speed occurred only during March). Wind strength is considered to be an important factor influencing spawning time because spawning during a period of slack water is likely to increase fertilisation success (Babcock et al. 1986). Therefore, a distinct period of low winds in October at the Bonaparte Archipelago could be advantageous for spawning during this period.

The data from this study suggests that conditions for reproduction in October/November are more favorable at the Bonaparte Archipelago than at the Dampier Archipelago, however sampling over more years is required to substantiate this. While it is possible that a combination of low SST and relatively strong winds could make conditions less favorable for reproduction in the Dampier Archipelago, additional data over a longer time period together with more detailed comparisons between the two locations is required to elucidate any strong conclusions. The spatio-temporal variability of coral spawning in Western Australia is only just beginning to be revealed, but offers much scope for further research into the factors constraining spawning time.

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Sexual Reproduction in the Soft Coral *Lobophytum* sp. in Tung Ping Chau Marine Park, Hong Kong SAR, China

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Abstract. The reproductive biology of soft corals in Hong Kong has never been investigated. The soft coral genus *Lobophytum* is a carpet-like encrusting coral and is commonly found in Tung Ping Chau Marine Park, Hong Kong SAR. Three samples from five marked *Lobophytum* sp. aggregations were collected monthly from 16 Aug 2006 to 7 Jul 2007. Histological sections of each sample were prepared and their gonadal development was observed under the microscope. From the histological analysis of the samples, all colonies bore only eggs. As *Lobophytum* was reported to be gonochoric, all the colonies sampled were therefore female. Male colonies may be missed during sampling. Oocytes from the samples measured increased in mean (±SD) diameter from 116.5±18.8 μm in Aug 2006 to 239.3±82.2 μm in Jun 2007, and dropped back to 93.0±23.2 μm in Jul 2007. This indicates that spawning of *Lobophytum* probably occurred in or before Jul 2007. The oocyte development appeared synchronized as shown in the simultaneous increase in their sizes. However, new oocytes likely developed before the release of the mature ones so that each cycle of development took more than one year to complete. Additional samples will be processed in order to confirm this initial observation.

Key words: *Lobophytum*, Reproductive Biology, soft coral, Hong Kong SAR, .

Introduction

Soft corals are one of the major components in coral reef worldwide aside from hard corals. They can increase the spatial heterogeneity in the reefs, which provide shelter to many other organisms (Jordan-Dahlgren 2002). Soft corals are rich in natural products (Blunt et al. 2005). They can protect and attack their neighbors and their bioactive compounds can be extracted for pharmaceutical and antifouling use. The structure of cemented large sclerites, called spiculite, at the basal part of *Sinularia polydactyla* can also contribute to reef-building (Schuhmacher 1997). Therefore, conservation of soft coral is an important issue, and studying its reproductive biology is one of the major steps to better understand its biology.

There are 29 species in 14 genera of soft corals in Hong Kong. They are mainly found in water from 3 to 20 m depth (Lee 2007). The number of investigations on soft corals in Hong Kong was limited. Previous studies examined their terpenoid contents (Zhang et al 1997; Zhang 2001), associated bacterial communities (Harder et al 2003; Dobretsov and Quan 2003) and distributions (Clark 1997; Fabricius and McCorry 2005; Lee 2007). No information on soft coral reproductive biology was available in Hong Kong.

The living range of soft corals is very wide. They are found from tropical to temperate regions and even in the poles, as well as from shallow water down to the deep sea (Fabricius and Alderslade 2001). There are very few studies about the biology and ecology of soft corals in the sub-tropical region, where Hong Kong is located. Having quite a high diversity of soft corals in such a small place like Hong Kong, it is worthy to investigate Hong Kong soft corals to help fill the information gap in this region.

The reproductive biology of the soft coral *Lobophytum* was the focus of this study. There were some previous studies on *Lobophytum* reproduction in Japan, Australia and Taiwan (Yamazato et al. 1981; Michalek-Wagner and Willis 2001; Fan et al. 2005). Such being the case, the results in this experiment can be compared with those from the other areas in order to help us understand more about the potential inducing factor of spawning in soft corals. This project also provided baseline information about the reproductive cycle of soft corals in Hong Kong, which is essential for their conservation.

Material and Methods

Site and species descriptions

The study site, Lung Lok Shui, is located in Tung Ping Chau Marine Park, Hong Kong SAR. Tung Ping Chau is a relatively remote island in NE Hong Kong. It was designated as a marine park in 2001 to protect the high richness of coral communities around the

island (Lee 2007). On the Northeastern side of Tung Ping Chau Marine Park, the shore is sheltered so that hard corals are abundant. In contrast, the southeastern side is more exposed such that soft corals and gorgonians are more common. Nine species of soft corals were found in Lung Lok Shui (Lee 2007).

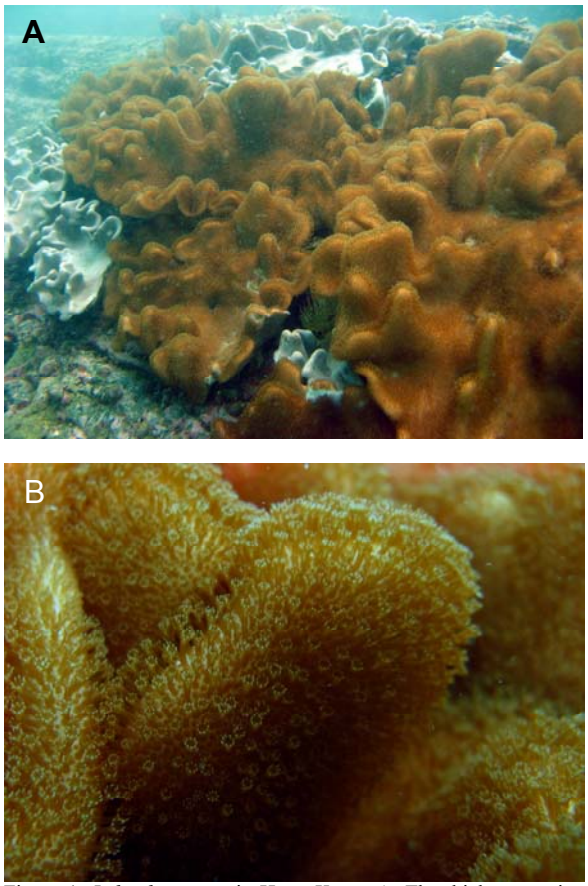

Figure 1: *Lobophytum*.sp. in Hong Kong: **A.** The thick-encrusting capitulum with lobes is yellowish-brown in color. **B.** Closed-up of capitulum showing the dimorphic polyps, in which only the autozooids bear gonads.

The genus *Lobophytum* is low-encrusting with a capitulum which bears polyps above its stalk (Verseveldt 1983). *Lobophytum* sp. is one of the most commonly found soft corals in Tung Ping Chau Marine Park. It is thick-encrusting with lobes and is yellowish-brown in color (Fig. 1). Being a zooxanthellate coral, it grows in shallower water, i.e., 6-11 m in Lung Lok Shui (Lee 2007). Sexual and asexual fissions were reported in *Lobophytum* (Fan et al 2005). It has dimorphic polyps, in which only autozooids but not siphonozooids bear gonads. They were reported to be gonochoric broadcaster (Yamazato et al 1981). Due to its relatively high abundance in the Marine Park, *Lobophytum* sp. was chosen to be the first soft coral in Hong Kong to be examined for its reproductive biology.

Collection and processing of the samples

Monthly sampling was done on *Lobophytum* aggregations in Lung Lok Shui. Five aggregations of *Lobophytum* were located in the site at 5-7 m depth. Assuming the colonies in the same aggregation to be derived from fission of a single colony, three small samples were cut from each aggregation each time. The samples were fixed in 10% formalin for 1 week and preserved in 75% ethanol afterwards. They were then decalcified and mounted in paraffin wax blocks. Thereafter, 7 μm thick microtome sections were prepared for gonad examination. Hematoxylin and eosin were applied to stain the samples.

The sections mounted on slides were observed under light microscope. Photos were taken for the six largest oocytes in each sample. Their longest diameter and the corresponding perpendicular diameter were measured using a computer program Image-Pro Plus 5.0©. The geometric diameter of each oocyte was calculated by the square root of the product of the two diameters. The monthly variation in the oocyte size was plotted with temperature to investigate if the spawning time is correlated with the temperature increase in summer.

Results

Only female gonads were found in the samples (Fig. 2a). This did not contradict with the previous reports that *Lobophytum* is gonochoric (Michalek-Wagner and Willis 2001), yet could not be supported by this result firmly. No planulae were observed in the coelenteron of the samples investigated. Therefore, *Lobophytum* sp. in Hong Kong should be a broadcaster. This is consistent with the previous studies on *Lobophytum* reported in other places.

The color of the oocytes changed from white to pale yellow as they matured (Fig. 2b). The oocyte sizes increased from 116.5±18.8 μm in Aug 2006 to 239.3±82.2 μm in Jun 2007, and dropped back to 93.0±23.2 μm in Jul 2007 as shown in Fig. 3 Corresponding water temperature in each sampling date plotted in the same figure illustrates the relationship between temperature and gonadal development. Spawning likely occurred between June and July 2007, at a time when the water temperature was possibly the highest in the year.

Discussion

Spawning of *Lobophytum* sp. in Hong Kong likely occurred in Jun-Jul 2007. Long day-light and high seawater temperature in summer may be the inducing factors for *Lobophytum* sp. to spawn. Previous studies on the reproductive biology of *Lobophytum* in other areas showed the spawning period to be in July to September for *L. pauciflorum* in Nanwan Bay, Taiwan (Fan et al. 2005), June for *L. crassum* in

Okinawa, Japan (Yamazato et al. 1981), and November for *L. compactum* and *L. crassum* in the Great Barrier Reef, Australia (Bowden et al. 1985; Michalek-Wagner and Willis 2001). All these reported spawning events worldwide occurred during summer and were probably related to the longer day-light time and/or the higher water temperature in this period. It may be an adaptive feature for the genus *Lobophytum* to spawn during summer. Warmer water temperature in summer may be more favorable for increasing the survivorship of the recruiting new coral larvae.

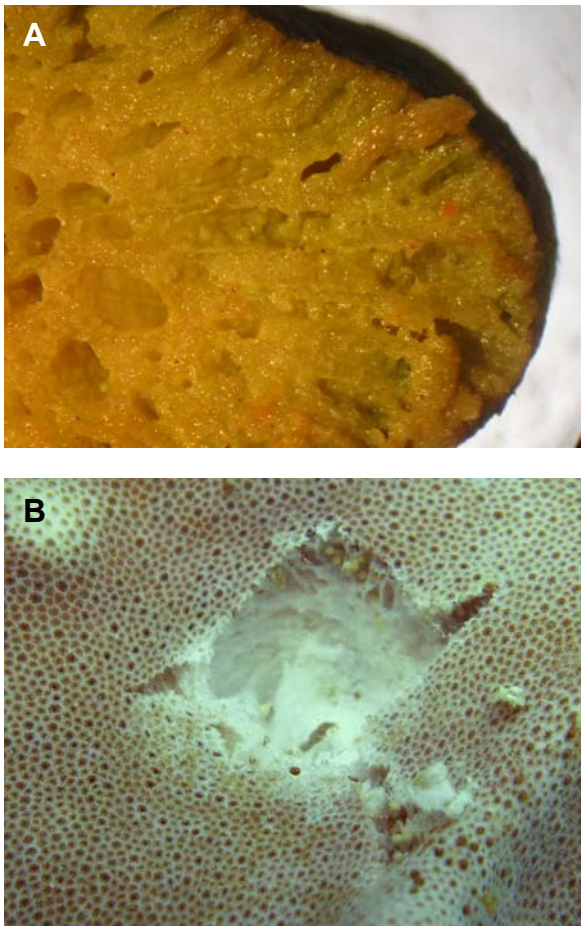

Figure 2: **A.** Section of the capitulum showing the female gonads and eggs. **B.** Color of the oocytes changed from white to pale yellow as they became mature.

Studies on reproduction pattern of hard corals in Hong Kong showed that *Platygyra acuta* (as *P. sinensis*) spawned in May (Liu and Ang 2002), *Favia speciosa* and *Favites abdita* in May-June, and *Leptastrea purpurea* in June-July (Lin 2003). All of these investigated hard corals also spawned in summer. The synchronized pattern of oocyte development of these soft and hard corals in Hong Kong appears to be under the control of these similar factors. Hong Kong is located near the northern limit of coral distribution. Low water temperature in winter (14°C) is not favorable for coral growth. It is thus likely that most soft and hard corals have adapted to spawn in summer to avoid the cold winter water temperature which may also be unfavorable for the early development of their recruits. More investigations are needed to confirm that this is indeed the general trend for other soft coral species in Hong Kong.

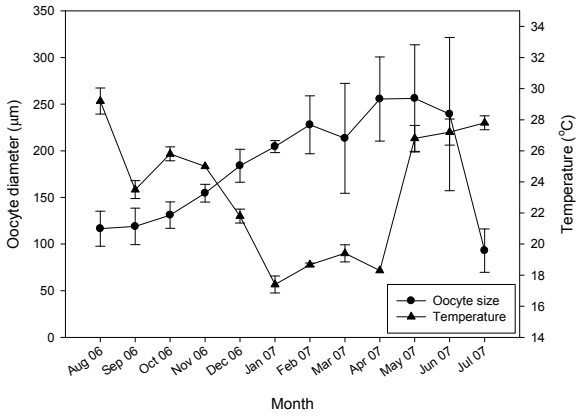

Figure 3: Changes in the mean size (±SD) of the oocyte over the sampling period. The corresponding mean (±SD) seawater temperature at each sampling date is also shown. Spawning of *Lobophytum* sp. likely occurred in Jun-Jul 2007.

The aggregations of *Lobophytum* sp. in Lung Lok Shui were sparsely distributed although there were plenty of uncolonized spaces in the substratum. In our study, only female colonies were found in this site. We hypothesize that some of these aggregated colonies were recruited from outside larval sources at the beginning, but the absence of male colonies would not allow more sexual recruits to be produced. Hence, there was no subsequent colonization of these free spaces by new recruitment. Asexual reproduction by fragmentation or fission becomes more important in forming separate dense aggregations to colonize the surrounding space. This hypothesis remains to be tested while we continue to search for the presence of male colonies in Lung Lok Shui as well as in other surrounding areas.

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Lunar periodicity of larval release and larval development of *Pocillopora damicornis* in Thailand

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Abstract. In this study, the lunar periodicity of larval release and development of larvae of *Pocillopora damicornis* in the Gulf of Thailand were investigated. Ten colonies of *P. damicornis*, approximately 15 cm in diameter, were tagged and collected for observation every month. To observe the releasing period of larvae, each colony was placed in a separate tank in a rearing system until the planula larvae were released. The results showed that *P. damicornis* released planulae during the day and night between 1-14 days after the new moon. However, the highest numbers of planula larvae were released 5-6 days after the new moon. The releasing rates were 0.19±0.05 larvae per polyp. Moreover, some colonies released larvae for up to 3 months consecutively. The newly released larvae were approximately 1 mm in length. The larvae started settling on a substrate within 0.5 hours after released, and depressed their body shape in 1.5 hours. The polyp, tentacle, and exoskeleton were observed after 40 hours. After 4 days, a juvenile coral (primary polyp) started budding and expanding its size. At the 6th month, the colony shape started uplifting.

Key words: *Pocillopora damicornis*, coral, lunar periodicity, larval release, larval development

Introduction

Pocillopora damicornis is typically a hermaphroditic brooder releasing the planula larvae with symbiotic zooxanthellae (Szmant 1986). However, in some areas such as Eastern Pacific, this species is a broadcast spawner (Glynn et al. 1991). Releasing periods are related to lunar cycle, which can vary in different areas (Harriott 1983; Richmond and Jokiel 1984; Chou and Quek 1993; Fan et al. 2002). For example, in Singapore, larvae of *P. damicornis* were released from a few days before the new moon to almost the first quarter (Chou and Quek 1993). In Southern Taiwan, larvae were released from lunar days 2-9 (Fan et al. 2002). After released, planula larvae of other pocilloporids settle rapidly on substrates (Baird and Morse 2004). They change their body shape, generate hard structures as a basal plate, and start budding after settling 3-7 days (Sato 1985; Babcock et al. 2003).

Although there are many papers on lunar periodicity and development of *P. damicornis*, some are contradictory in releasing periods, and none of them are from Thailand (Harriott 1983; Richmond and Jokiel 1984; Sato 1985; Chou and Quek 1993; Fan et al. 2002; Babcock et al. 2003; Baird and Morse 2004). The purpose of this study is to investigate the larval releasing period and development of *P. damicornis* in Thailand. The releasing rate and settlement behavior of larvae are also observed.

Materials and Methods

To observe the releasing period of larvae of *P. damicornis*, 10 colonies (each colony 15 cm in diameter) were randomly collected at Khao Maa Cho, Ko Pla Muk and Ko Samae San in Sattahip area, Chonburi Province (Fig. 1). Then, they were tagged and brought back to the laboratory to observe during the full moon and new moon of each month from March 2006 to December 2007. In the laboratory, each colony was placed in a separate tank in a rearing system until the planula larvae were released. To determine numbers of releasing larvae, the larvae were collected from each tank using a Pasteur pipette, and counted every day from the beginning to the end of the releasing periods.

In addition, the releasing larval rates were investigated. Three fragments of corals approximately 3-4 cm in length were randomly cut from each of 5 colonies. Then, the numbers of releasing larvae were counted in each fragment. Each fragment was further decalcified using formic acid solution and sodium citrate to determine the numbers of polyps. Then, numbers of released larvae were compared on a polyp basis.

After the larvae settled, their development stages were observed under the stereo-light microscope. The behavior during the settlement and metamorphosis were also observed.

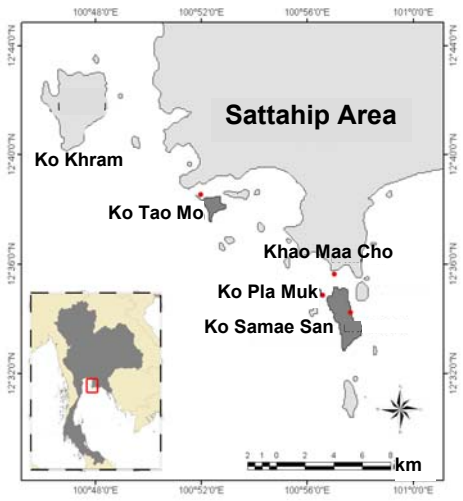

Figure 1: Study sites at Khao Maa Cho, Ko Pla Muk and Ko Samae San, Chonburi Province, where *P. damicornis* was collected

Results

Planulae size of *P. damicornis* was approximately 1 mm. Planulae were released almost every month during 1-14 days after the new moon (Fig. 2). However, the peak of larval release occurred 5-6 days after the new moon. The average numbers of larvae released ranged between 107.2-457.2 individuals per colony per day (Fig. 3). Larvae of *P. damicornis* were found every month. The average numbers of larvae released was high in September 2006 and May 2007. However, when using statistical analyses, there was no significant difference in the numbers of larvae released between months (p>0.05). In addition, the results showed that some colonies could release planulae for up to 3 months consecutively. However, other colonies released planulae every other month. From the observation and calculation, the rate of releasing *P. damicornis* larvae was 0.19±0.05 individuals per polyp.

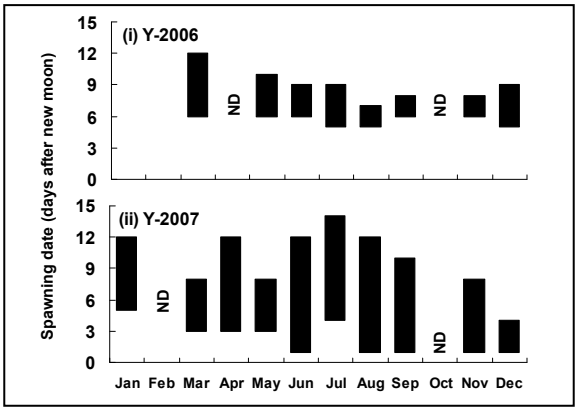

Figure 2: Releasing periods of *P. damicornis* larvae related to lunar cycle during March 2006 to December 2007 (ND: no observation)

After released, the larvae started settling within 0.5 hours. They compressed their body within 1.5 hours, and changed their shape to barrel shape (Fig. 4). During the experiments, larval behaviors were observed. Those behaviors included 1) touching substrates and then swimming (termed touch down), 2) creeping on surfaces of substrates, and 3) spinning while touching substrates. The metamorphosis of *P. damicornis* larvae was shown in Fig. 4. Within 20 hours, their exoskeletons fused to the substratum. The polyp, tentacle, and exoskeleton were observed after 40 hours. A primary polyp started budding to several new polyps 4 days after settling.

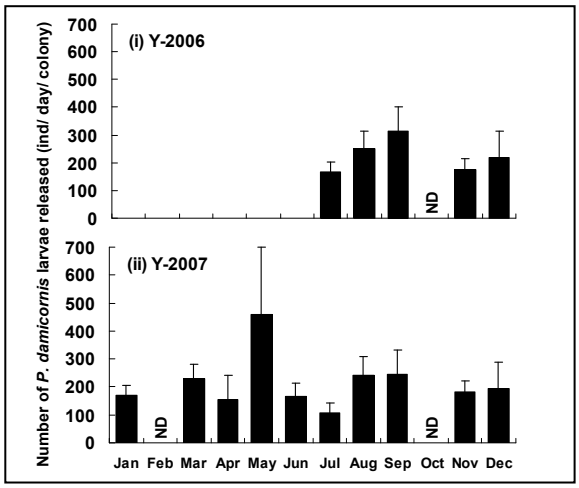

Figure 3: Average number (± SE; n=10) of *P. damicornis* larvae released daily during July 2006 to December 2007 (colony size: 15 cm in diameter) (ND: no observation)

Discussion

In this study, *P. damicornis* in Thailand was found to release planulae during the day and night between 1-14 days after the new moon. In Taiwan and Australia, the releasing periods of *P. damicornis* larvae were between 2-9 and 1-15 days after the new moon respectively (Richmond and Hunter 1990; Fan et al. 2002), while in Singapore, it was between a few days before the new moon to almost the first quarter (Chou and Quek 1993). In agreement with other study (Hodgson 1985), *P. damicornis* in Thailand also released both during the day and at night. From the observation, there was no difference in the numbers of larvae released between months (p>0.05). The results also showed that planulae were present every month. In Heron Island, Australia, Tanner (1996) reported that *P. damicornis* larvae were observed during summer months (between September to April), but were rarely present during the winter. Harriott (1983) showed that the reproduction of *P. damicornis* was not synchronized within a colony due to different stages of gonad maturation in different polyps. Thus, every polyp in a colony may not release larvae during

a certain reproductive cycle (Harriott 1983). In addition, Fadlallah (1983) suggested that in *P. damicornis,* testes matured before ovaries to avoid self-fertilization, which led to asynchronization in a colony.

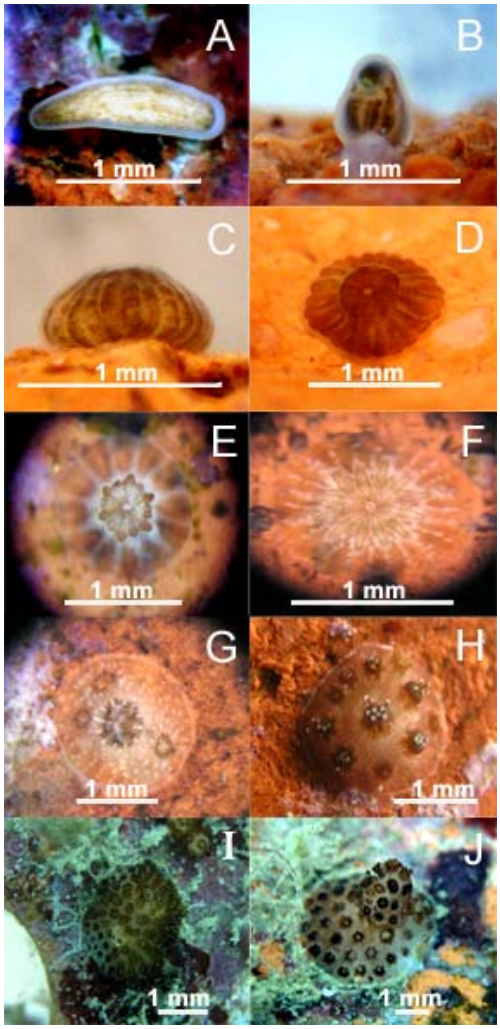

Figure 4: Metamorphosis of *P. damicornis*. A) searching for suitable plate; B) started attaching, 0.5 hrs; C) depressed its shape, 1.5 hrs; D) polyp formation, 5 hrs; E) tentacles and exoskeleton formation, 20 hrs; F) tentacles and exoskeleton observed, 40 hrs; G) started budding, 4 days; H) 1 month old; I) size expanding, 3 months old; J) started uplifting, 6 months old.

The rate of larval release can vary depending on colony sizes (Tioho et al. 2001). In this study, rate of releasing *P. damicornis* larvae was 0.19±0.05 individuals per polyp per month. Tanner (1996) showed that *P. damicornis* in Heron Island, Australia released 1-3 larvae per polyp, and in each month, only 1/3-1/2 of all polyps in a colony released planulae.

The settlement behaviors and development of larvae observed in this study were similar to other previous studies (Sato 1985; Babcock et al. 2003; Baird and Morse, 2004). Once larvae settled, metamorphosis was started and basal plate was formed. *P. damicornis* started budding 3-7 days after the settlement (Sato 1985).

In conclusion, lunar periodicity of larval release by *P. damicornis* in Thailand was consistent with those reported in other areas. However, the releasing period in Thailand was longer than that of other areas. More studies are needed to determine what environmental factors can influence the gonad maturation of *P. damicornis* in Thailand.

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Light sensing and the coordination of coral broadcast spawning behavior

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Abstract. In the Caribbean and Gulf of Mexico hermatypic corals undergo mass spawning on just one or two nights per year, peaking on the eighth evening after the August full moon. This event occurs with extraordinary consistency and is predictable to within a few minutes from year to year. Corals use the moon to determine the date of spawning and sunset time to set the hour and minute of gamete release. We have been exploring whether these two parameters entrain biological rhythms or whether corals respond directly to environmental signals. The literature supports a potential role for entrained circalunar systems setting the spawn date and for non-entrained direct responses to light setting the spawn time but neither has been conclusively demonstrated to date. We propose that G-protein coupled photoreceptors modulate second messenger levels in the cytoplasm and that these in turn lead to changes in protein abundance or protein phosphorylation patterns which then control spawning behavior. In support of this hypothesis we have identified dozens of differences in the proteome of Montastraeacavernosatissue dependent on light. We are using these molecular markers to dissect the cellular basis of lunar and solar light responses.

Key words:circadian rhythms, lunar periodicity, broadcast spawning, mass spawning, scleractinian

Introduction

Broadcast spawn timing is regulated by multiple environmental cycles- seasonal, lunar and solar. The first of these, seasonal, sets the month of spawning in each locale. How exactly corals synchronize spawning with seasonal cycles of gonad maturation remains unknown, but it is associated with solar insolation patterns (van Woesik et al. 2006) and periods of calm weather (Hagman et al. 1998). The second environmental parameter important in spawn timing is the lunar cycle, which sets the date of spawning(Babcock et al. 1986). This is clearly illustrated by the accuracy with which spawning dates can be predicted using the lunar calendar. The third parameter is the solar cycle, which sets the hour and minute of spawning (Levitan et al. 2004; Vize et al. 2005). This is clear from the observation that spawn time changes with the time of year and changes in day length and can be predicted to within minutes relative to sunset time from year to year.

Each of these cycles- seasonal, lunar and solar- could directly regulate cellular processes in corals or could act by entraining biological rhythms with annual, lunar or daily periodicities, and the entrained rhythms would then regulate the processes. As little is known about how the month of spawning is set, we will focus on the date and time of broadcast spawning for the remainder of this report. Excellent evidence exists for the presence on circalunar rhythms in reproductive behavior in brooding corals. Planula release by brooders can continue for many months even when the lunar input is blocked or is held constant (Jokiel et al., 1985). Furthermore, monthly cycles of planula release takes months to be reestablished when placed in a lunar phase shifted regimen, once again strong evidence that entrainment is involved (Jokiel et al., 1985). An entrained system would have considerable advantages in keeping spawning dates consistent from year to year as fluctuations in weather, cloud cover etc. would have minimal effects on timing.

While corals display some circadian rhythms with a periodicity of around 24 hours (Sweeney, 1976) these do not appear to regulate the time of spawn release. When sunset time is artificially moved forward on the day of spawning, spawn release occurs in a correspondingly early manner (Knowlton et al., 1997; Levitan et al., 2004), which would not happen if an entrained system regulated this process. This indicates that either light directly inhibits or darkness directly promotes spawning behavior. Species species that spawn in different time windows must have different thresholds for a common light regulated factor (e.g., a second messenger) or the factor changes at different rates in different species. By determining what signal transduction pathways

are involved and what second messengers they use will allow us to uncover how corals perceive light and to understand the molecular control of coral spawning behavior.

Material and Methods

Spawning observations

The documentation of spawning times at the Flower Garden Banks, northwest Gulf of Mexico, have been made by our research group for the past 12 seasons. The detailed observations from 1997-2003 were compiled in a recent report (Vize et al., 2005) and are also summarized below. Common species at the FGB that were surveyed include: Colpophyllianatans (6%), Diploriastrigosa (13%), Montastraeaannularis (<6%), M. cavernosa (13%), M. faveolata (9%), M. franksi (37%) and Stephanocoeniaintersepta (<6%), where the percentage of live coral cover indicated in brackets if it is known (Pattengill-Semens and Gittings, 2003).

Tissue sampling

Fragments were collected from a single M.cavernosa colony at noon and kept on deck in a bucket of seawater along with an air-stone and illuminated by ambient light. Tissue was collected from coral fragments by scraping the surface layer into a test tube containing a commercial protein preservation buffer (phosphosafe- Novagen). A second sample was treated in an identical manner but harvested 4.25 hours post-sunset (midnight).

Protein analysis

Proteins were visualized by 2D electrophoresis followed by silver staining using standard protocols Marengo et al., 2008). The first dimension was a pH 3 – 10 IEF gradient and the second dimension by molecular weight (10% polyacrylamide).

Results

Spawn timing

Observations of spawn timing were made by the same group of divers each year. This was found to greatly increase the consistency of spawn timing reports (Vize et al., 2005). Spawn times for each of the six common species that broadcast spawn on the eighth evening after the August full moon are shown in Fig.1. Spawning windows for each species are approximately 30 minutes in length except for D.strigosa, which is due to a slow tailing off in spawning activity in this coral. However, like other species, the peak of D. strigosa spawning also lasts for around 30 minutes. Fig.1 also highlights the unique time windows in which each species spawns, once again with the single notable exception of D.strigosa.

In both gonochoric species surveyed the males colonies begin to release sperm approximately 20 minutes prior to the time at which females begin to expel eggs. We have hypothesized that while females may be primed and ready to release via similar timing processes as males, sperm may act as the ultimate trigger of egg release(Hagman et al., 1998). In the case of the common and large species, M.cavernosa, females spawn for approximately 30 minutes. For the less common and much smaller species S. intersepta, spawning is much briefer and females all release within a window lasting only about 10 minutes.

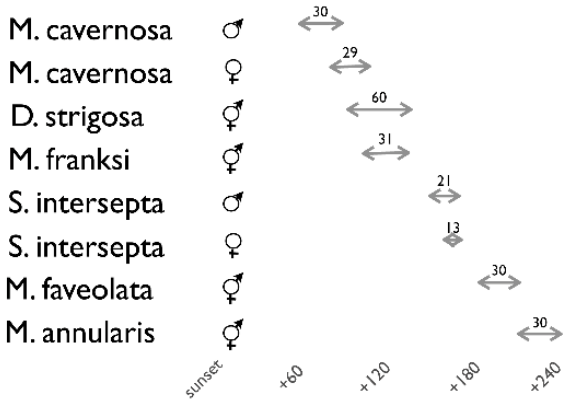

Figure1: FGB spawn times relative to sunset. The number above the arrow indicates the length of the spawning window in minutes. Time after sunset is indicated on the X axis.

This pattern is extremely consistent from season to season. Figure 2 shows the average start and stop times for each species, averaged over eight spawning events, plus the variance over the eight seasons. Of the 16 times noted, 10 are consistent to within 15 minutes over all eight seasons, and 8 consistent to less than 10 minutes.

		start	+/-	stop	+/-
M. cavernosa	♂	1:01	4	1:31	13
M. cavernosa	♀	1:27	23	1:56	9
D. strigosa	☿	1:29	21	2:29	21
M. franksi	☿	1:51	16	2:20	19
S. intersepta	♂	2:38	13	2:59	7
S. intersepta	♀	2:46	10	2:59	7
M. faveolata	☿	3:13	7	3:43	7
M. annularis	☿	3:42	6	4:12	6

Figure 2: Seasonal consistency in coral spawning times. The average time at which coral spawning begins, along with the variation from the average for each species/sex is indicated.

The number of individual corals spawning in any particular season varies quite widely at this location.

Light sensing

Other invertebrates sense light via opsin and rhodopsin visual photoreceptors and non-visual melanopsins (Terakita et al., 2005). In all cases the photoreceptors act via trimeric G-proteins and regulate levels of a soluble second messenger within the cytoplasm of the light-responsive cell (Rayer et al., 1990). If the temporal precision of coral spawn timing is regulated by the same system, as it is likely to be, light may act by upregulating a second messenger that is an inhibitor of spawning or darkness may allow the accumulation of a second messenger that activates spawning. In bothscenarios the altered second messenger levels will lead to changes in protein phosphorylation patterns and the changes in protein activity caused by these changes could trigger spawning. Similar universal systems regulate cellular processes in examples ranging from bacterial chemotaxis to human neurobiology and are direct cellular responses to environmental stimuli (Bourne, 2006).An alternative to this class of processes is entrained biological rhythms (Fig.3)(Dunlap et al., 2004). While light still acts on cells in the same manner and causes changes in second messengers and protein phosphorylation, these changes entrain biological clocks over a period of time. An entrained clock could then accurately regulate cellular processes even when the entraining stimulus is removed (Dunlap et al., 2004).

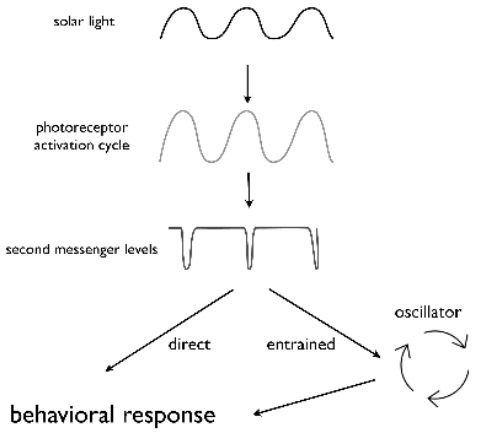

Figure 3: Coral timing may be directly controlled by environmental signals or it may be indirectly regulated by an entrained biological rhythm.

If the spawning response is direct, changes in second messenger levels will control spawn timing while if an entrained clock regulates spawning the key molecules may be regulated at the transcriptional or protein stability levels. There is considerable overlap between these options, but in each case a different set of mediators is actually regulating the biological process.

We have initiated an analysis of changes in protein abundance associated with changes in

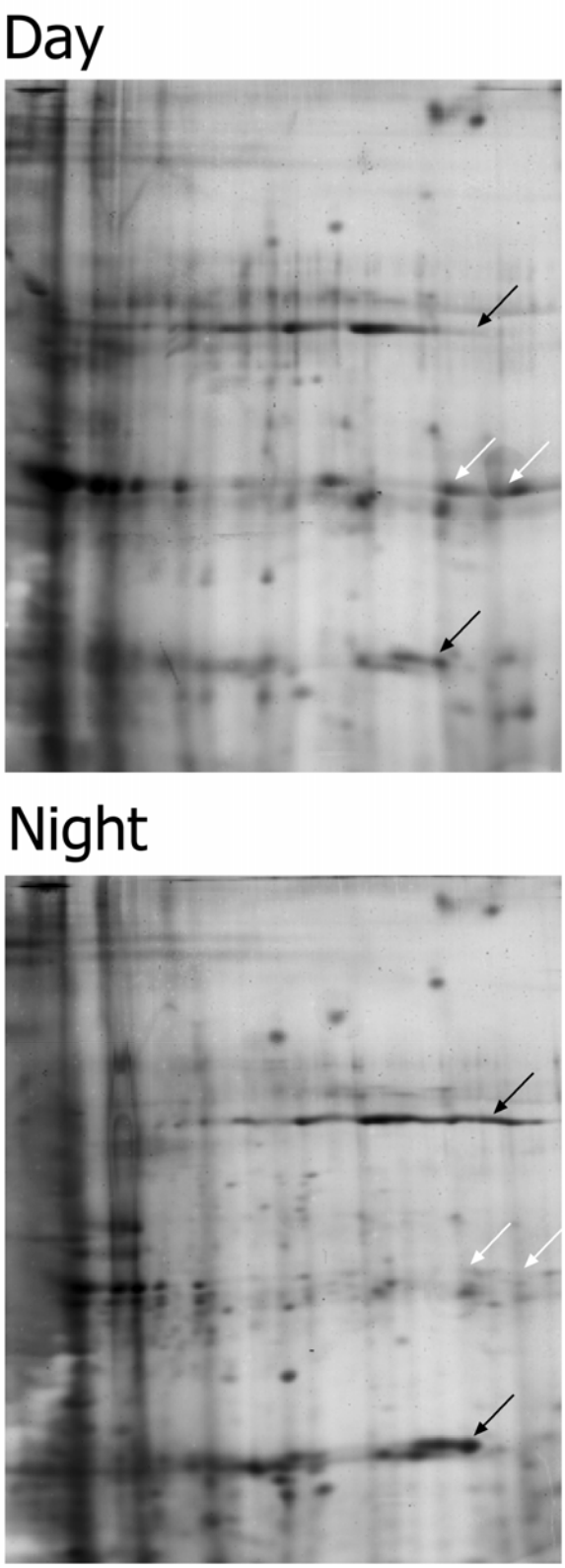

Figure 4: Proteomic analysis of coral tissue harvested in the day or at night. White arrows indicate examples of proteins more abundant in the day samples and black arrows indicate proteins more abundant in darkness. IEF in dimension 1 was from 3 (left) to 10 (right). Molecular weight runs from high (top) to low (bottom).

coralillumination using 2D electrophoresis. Preliminary analysis indicates that dozens of proteins differ in the proteome in M. cavernosa under different lighting conditions (Fig.4).

Discussion

Caribbean corals display extraordinarily consistent spawning behavior, varying by only a few minutes in the time at which they begin and end spawning from season to season. Evidence in the literature implicates circalunar systems as playing a central role in regulating the date of spawning (Jokiel et al., 1985), but the system controlling the time of spawning appears to be a direct response to the environment (Knowlton et al., 1997). However, definitive evidence for direct versus entrained regulation of spawning time and date has not been generated.

Analysis of proteins from the same individual coral collected at midday or midnight indicates that there are many differences in the proteome over this time. As this analysis was performed on whole tissue homogenates containing both coral cells and zooxanthallae, identified proteins could belong to either organism, or to other symbiotic microorganisms. However, as the vast bulk of the protein is from coral cells these are the most likely to be visualized by silver staining.

Although the identity of the proteins that change in abundance from day to night is not known, these can be used to test many aspects of coral responses to light. For example, if a protein is present in the day sample but not the night sample, we can test whether this change still occurs if coral tissue is kept under constant illumination or constant darkness. If it continues to cycle when environmental parameters are constant this must be due to an entrained biological clock, while if it remains constant under constant conditions, it must be directly regulated. We can also test whether pharmacological agents that stimulate or block specific signal transduction pathways can convert light type protein patterns to dark type, and visa versa. Finally, protein gels such as these can be transferred to membranes and probed with phosphospecific antibodies to study changes in the phosphoproteome, determine if these are entrained or direct, and whether they respond to signal transduction pathway changes. This course of experiments will allow us to determine the biochemical basis of the temporal regulation of coral spawning behavior.

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The effects of habitat on coral resistance and resilience to bleaching

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Abstract
Coral bleaching related to climate change is one of the major threats to coral reefs today. This study examines bleaching responses of scleractinian corals at four Kenyan sites (Kanamai, Vipingo, Mombasa and Nyali) representing shallower and deeper lagoon habitats. Bleaching incidence was monitored for the whole coral community, while zooxanthella densities and chlorophyll levels were monitored for selected species (*Pocillopora damicornis*, *Porites lutea* and *Porites cylindrica*) during non-bleaching and mild-bleaching years. The objective was to determine whether corals in different habitats display varying resistance and resilience to bleaching and to indicate which habitat characteristics are responsible. Differences in bleaching responses between shallower and deeper lagoons were observed, with shallower sites Kanamai and Vipingo exhibiting lower bleaching incidence than deeper sites Nyali and Mombasa. Shallower lagoons also displayed higher fluctuations in light and temperature than deeper sites, with higher maximums, lower minimums, higher standard deviations and higher diel variation. This suggests that corals in the shallower lagoons have acclimatized to become more resistant to bleaching because of the fluctuating conditions they endure daily. In sites with higher bleaching incidence, it was found that coral recovery occurred more quickly in the protected area (Mombasa) compared to the reserve (Nyali).

Keywords
Coral bleaching, coral acclimatization, resilience, Kenya

Introduction

Coral colonies have been observed to acclimatize or adapt to fluctuating temperature and irradiance levels, and this prior experience might make the colonies more able to resist unexpected bleaching events (Brown et al. 2002a, Brown et al. 2002b). Acclimatization can be defined as the ability of an organism to undergo phenotypic changes in response to stress in the natural environment that result in the readjustment of the organism's resistance levels to that stress (Coles and Brown 2003). Adaptation occurs over the long-term when the more stenotopic members of a population are eliminated by environmental stress, leaving the more resistant organisms to reproduce and recruit to available habitat (Coles and Brown 2003). Threshold temperatures that induce coral bleaching-related mortality vary worldwide due to selective adaptation and according to the maximum water temperatures that are normal in the area, implying a capacity of corals and/or zooxanthellae to survive higher temperatures for a certain period of time. Furthermore, corals that are regularly exposed to stressful environmental conditions have been shown to acclimatize and exhibit physiological resistance to elevated temperatures and UV-radiation that exceed normal thresholds (Brown et al. 2000; Brown et al. 2002a; Brown et al. 2002b, Coles and Brown 2003).

In order to combat the worst effects of climate change and to conserve this valuable ecosystem, it is important to determine which factors affect coral reef bleaching resistance and resilience and to apply this knowledge in management plans. Although bleaching events cannot be prevented by managers, by implementing appropriate management responses it is possible to help a coral reef recover from bleaching and to mitigate the worst effects.

This study examines the bleaching responses of corals at four sites (Nyali, Mombasa Marine Park, Kanamai and Vipingo) representing two distinct lagoon habitats on the Kenyan coast (deeper and shallower lagoons). The sites were characterized using environmental parameters such as depth, water flow, light and temperature. Bleaching responses were monitored for the general coral community and zooxanthella densities and chlorophyll levels were monitored for target species (*Pocillopora damicornis*, *Porites lutea* and *Porites cylindrica*)

during a non-bleaching year (2006) and a mild bleaching year (2007). The objective of this study is thus to determine whether corals in different habitats display different bleaching responses (i.e. resistance and resilience) and to indicate which environmental characteristics are responsible for the variation in response.

Materials and Methods

Five large and healthy coral colonies for each target species (*Pocillopora damicornis*, *Porites cylindrica* and *Porites lutea*) were mapped in two shallower lagoons (Kanamai and Vipingo, 0.4 m depth and 0.6 m at low tide respectively) and two deeper lagoons (Mombasa Marine Park and Nyali, 1.4 m and 1.8 m depth at low tide respectively) along the Kenyan coast. Target species were chosen according to general abundance and bleaching susceptibility, with one highly susceptible (*Pocillopora damicornis*), one moderately susceptible (*Porites cylindrica*) and one resistant (*Porites lutea*) target species. Coral size class and line intercept transect data were also collected for two fixed 25x2 m transects at each site.

The bleaching season in Kenya occurs during the late northeast monsoon from mid March when doldrum conditions become most intense to late April when the first storms of the southeast monsoon induce cooling of surface waters. In the two years of the study, sampling began before bleaching during the northeast monsoon (February and early March), continued during bleaching season of the northeast monsoon (late March and April) and finished during the recovery period of the southeast monsoon (May, June and July).

Coral fragments were collected from the mapped colonies using a hammer and chisel. The fragments were transported to the laboratory submerged in seawater in small plastic bottles and were held in an aerated seawater tank for less than 24 hours. Coral tissue was removed from the skeleton using a water jet. Seawater was then added to the tissue to make up a fixed volume of 500 ml. The tissue slurry was homogenised using a hand-held homogenizer and 1.0 ml of homogenate was loaded into a Sedgwick-Rafter chamber. Using a compound binocular microscope and a magnification of x400, the number of zooxanthellae in 10 random quadrats was recorded. The counting chamber was then reloaded and another 10 random quadrats were counted. The area of the coral skeleton was measured using the aluminium foil method (Marsh 1970); the area/weight ratio of aluminium foil was determined and coral skeletons were then wrapped in this foil. The foil was then trimmed to fit the skeleton area and weighed. Using the weight of the trimmed foil and the area/weight ration for the foil, the skeleton area could be determined.

To measure chlorophyll *a* levels, a known volume of the homogenate was filtered through a GF/F filter paper and the filtrate was dissolved in 10 ml of 90% acetone. It was then centrifuged for 10 minutes at 2000 rpm and the chlorophyll absorbance was read using a Whatman spectrophotometer.

If it was observed, bleaching incidence was recorded in the water using a random swim method. This consists of noting the size class, genus and bleached/pale/dead percentages of each bleached colony in 20 haphazard 2 m^2 areas at each site.

Water temperature was monitored using underwater Hobo temperature loggers that were left at each site during the entire duration of the study. Light was monitored using a Hobo light meter for only 24-hour periods on the same day and time at each site to avoid theft and overgrowth of the light meter. Water flow was measured using a clod card method (McClanahan et al. 2005). Plaster-of-Paris clod cards were made in an ice-cube tray, dried and weighed before being deployed in the field in small net bags that were tied to the substrate. They were collected 24 hours later, dried and weighed again in order to determine how much had dissolved. Current speed in g d^{-1} (24 h) was obtained by dividing weight loss by deployment time. Weight loss [g] was then converted to flow speed (Vw = cm s^{-1}) using the equation $Vw = (DWclod - 0.932)/2.357$, which was obtained by studies of clod dissolution in a flume with controlled water flow (Anzai 2001).

Statistical analyses were performed using the program Minitab (version 10.1). Differences in zooxanthella density were investigated using the Man-Whitney U test with Bonferroni corrections and the Kruskal-Wallis test.

Results

Depth

Nyali and MMP are deeper lagoons (1.8 m depth and 1.4 m depth at mean low tide respectively) while Kanamai and Vipingo are shallower lagoons (0.4 m depth and 0.6 m at mean low tide respectively).

Temperature

The shallower lagoons Kanamai and Vipingo displayed the most fluctuating temperature conditions, with higher standard deviations (±1.9°C and ±1.5°C respectively), higher maximum temperatures (36.2°C and 33.8°C respectively), lower minimum temperatures (24.0°C and 23.7°C respectively) and bigger differences between diurnal and nocturnal temperatures (+0.9°C and +0.4°C respectively) than the deeper lagoons. Between the

two, Kanamai displayed larger fluctuations, as well as the highest average temperature overall (27.8°C) .

The deeper lagoons of MMP and Nyali displayed less pronounced temperature variations with lower standard deviations (both ±1.4°C) as well as smaller maximum-minimum (+8.9°C and +8.1°C respectively) and diurnal-nocturnal (both +0.2°C) differences than the shallower lagoons. However, the deeper lagoons displayed bigger temperature increases from the 2006 to 2007 northeast monsoon bleaching seasons, with a +0.9°C increase in Nyali and a +0.8°C increase in MMP compared to a +0.7°C increase in both Kanamai and Vipingo (fig. 1).

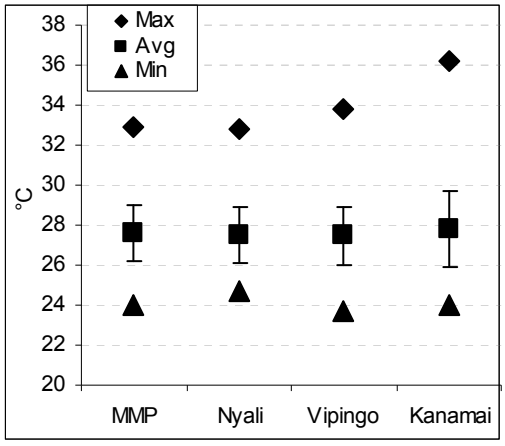

Figure 1.
Average, standard deviation, minimum and maximum temperatures for all sites.

Light

As expected, the shallower lagoons Kanamai and Vipingo also displayed more fluctuating light regimes than the deeper lagoons Kanamai (4913±14,315 lux) and Vipingo (4758±10,489 lux) both displayed much higher average light levels, higher standard deviations and higher maxima (70,846 lux and 110,223 lux respectively) than MMP (average of 518±879 lux, maximum of 5167 lux). Although light data are unavailable for Nyali, it is likely that light levels are similar to MMP given the depth and sediment levels of the site.

Water flow

There was no clear trend between water flow at deeper and shallower lagoons. MMP consistently displayed the highest average water flow during both neap (6.4 cm/s) and spring tide cycles (9.0 cm/s). Kanamai (5.4 cm/s) displayed similar water flow to Nyali (5.5 cm/s) and Vipingo (5.6 cm/s) during neap tide and it appears that MMP stands out as the site displaying highest water flow.

Substrate cover

MMP and Kanamai displayed higher hard coral cover (28.4% and 23.0% respectively) while Nyali and Vipingo displayed lower hard coral cover (14.7% and 10.6% respectively). Macroalgal cover was higher in deeper sites MMP (25.5%) and Nyali (21.0%) than in shallower sites Vipingo (8.3%) and Kanamai (3.7%).

Coral cover

All sites are dominated by massive and branching *Porites* colonies. 90.5% of MMP, 80.3% of Kanamai, 59.9% of Nyali and 42.2% of Vipingo coral cover was massive and branching *Porites.* The highest relative coral cover of the more bleaching-susceptible genera *Acropora* and *Pocillopora* is found in Vipingo (10.2%) and Nyali (8.3%). *Acropora* and *Pocillopora* only accounted for 3.7% of Kanamai's coral cover and 0.7% of MMP's coral cover.

Bleaching and mortality incidence

Bleaching was first observed during late April of 2007. The shallower lagoons Kanamai and Vipingo exhibited lower levels of combined paling, bleaching and mortality (4.4% and 6.3% of total coral area respectively) than the deeper lagoons Nyali and MMP (35.1% and 26.9% of total coral area respectively). During the recovery period, MMP still exhibited relatively high bleaching incidence (9.8%) but low mortality (0.9%). Nyali exhibited the highest mortality (11.7%) and also relatively high bleaching (4.5%). In July, mortality increased to 13.1% in Nyali and 2% in MMP, but bleaching incidence in MMP decreased to 6.0%, indicating recovery (fig. 2).

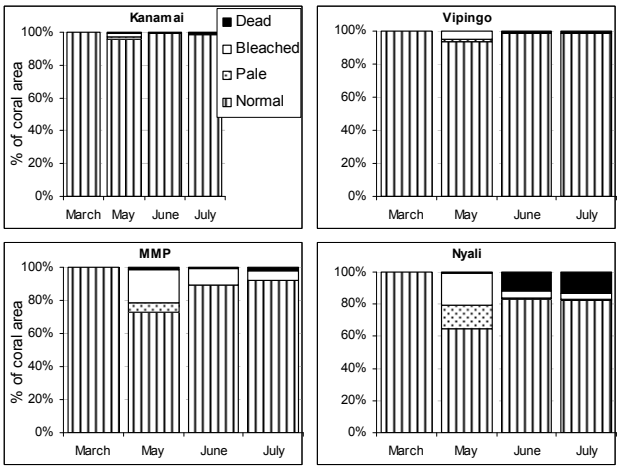

Figure 2. Bleaching and mortality incidence for all for sites from March to July 2007. Nyali and MMP exhibited higher bleaching than Kanamai and Nyali. Corals in MMP recovered better than in Nyali.

Pocillopora and *Porites* accounted for the highest proportion of bleached colonies, but differed greatly

in their mortality rates. In MMP, Nyali and Vipingo, *Pocillopora* accounted for 37%, 47.1% and 33.3% of bleached colonies respectively. In Kanamai, MMP and Nyali, *Porites* accounted for 47.2%, 28.3% and 13.8% of bleached colonies respectively. However, during the recovery period of June and July, *Porites* experienced low bleaching-related mortality while *Pocillopora* experienced highest mortality in all sites (from 37.5% in Vipingo to 90% in MMP). Of the three target species only *Pocillopora damicornis* suffered significant mortality in both 2006 (50% overall mortality) and 2007 (47% overall mortality) with colonies in Kanamai experiencing the lowest mortality rate in both years.

Zooxanthella densities

Average zooxanthella densities for marked colonies (*Pocillopora damicornis*, *Porites lutea* and *Porites cylindrica*) were higher for all species at all sites in 2006 than in 2007 (Man-Whitney U test, $p<0.05$). Average zooxanthella densities were also higher in shallower sites than in deeper sites for all species (Man-Whitney U test $p<0.001$). In general, Kanamai exhibited the highest average zooxanthella densities while MMP displayed the lowest. *Porites lutea* colonies exhibited the highest zooxanthella densities while *Pocillopora damicornis* (Kruskal-Wallis, $p<0.001$).

Chlorophyll a levels

Chlorophyll *a* concentrations were generally higher in shallower than in deeper sites, and Kanamai exhibited the highest chlorophyll *a* concentrations while MMP exhibited the lowest (Man-Whitney U test, $p<0.05$).

Discussion

Considerable differences in bleaching responses between shallower and deeper lagoon sites were observed, with shallower sites Kanamai and Vipingo exhibiting much lower bleaching incidence than deeper sites Nyali and MMP in both haphazard sampling and marked colonies. These results suggest that corals in the shallower lagoons have acclimatized and/or adapted to the more fluctuating environmental conditions they endure on a daily basis and have become more resistant to thermal stress than corals in the deeper lagoons. McClanahan et al (2005) found similar responses and concluded that 'that benign or stable environments will produce communities that are less resilient to rare climatic disturbances but if disturbances are sufficiently infrequent, they will maintain high numbers of species. Variable or stressed environments will maintain dominant organisms capable of withstanding climatic disturbances but will lose those rare members of the community that cannot adapt to the stressful conditions.'

Shallower sites Kanamai and Vipingo displayed more fluctuating temperature and light regimes than deeper sites Nyali and MMP with higher maxima, lower minima, larger standard deviations and larger diel variations. The smaller volumes of water in the shallower lagoons probably explain the difference in temperature and light regimes; shallower lagoons absorb but also lose heat more quickly and attenuate less light than in the deeper lagoons.

Differences in temperature and light regimes could also explain differences in bleaching responses between similar-depth sites. Kanamai displayed lower bleaching incidence and bleaching-related mortality than Vipingo and is also a shallower site with higher average temperatures, standard deviations, differences between maximums and minimums, diel variations, maximum light levels and standard deviation in light levels. Similarly, MMP displayed higher average temperatures and difference between maximum and minimum temperature than Nyali and also suffered lower bleaching incidence,

On the other hand, water flow did not correlate well with bleaching incidence, and although corals in Nyali suffered much higher bleaching incidence than Kanamai and Vipingo, all these sites displayed very similar water flow velocities. It therefore appears that light and temperature histories were more influential than water flow in determining the bleaching responses of corals.

Studies have shown that corals with higher zooxanthella densities are more resistant to bleaching (Grimsditch et al. 2008), a hypothesis that is further confirmed by this study. *Porites lutea* displayed the highest densities and was the most resistant to bleaching. *Porites* colonies made up a large proportion of bleached corals but they exhibited very little mortality. *Pocillopora damicornis* exhibited the lowest densities and was the most susceptible to bleaching, accounting for most bleaching-related mortality in all sites. Zooxanthellae population dynamics could thus partly explain bleaching responses of different genera.

All sites were dominated by massive and branching *Porites* corals, discounting the possibility that large variations in coral community compositions determined bleaching responses. However, differences in community composition could partially explain varying bleaching responses between similar-depth sites. Nyali exhibited higher bleaching incidence than MMP and also a higher proportion of area covered by bleaching-susceptible

genera (i.e. *Pocillopora* and *Acropora*). The same applies to Vipingo compared to Kanamai.

During the recovery period, colonies in Nyali displayed higher mortality than those in MMP, while a higher proportion of corals in MMP remained bleached but did not die. It is possible that colonies in Nyali were being overgrown by algae more quickly than those in MMP, which remained in a bleached state longer. This could be due to the protected status of MMP and that herbivory levels could be higher than in Nyali, which is subject to high fishing pressure. Populations of important herbivores such as parrotfish and surgeonfish have been shown to be significantly higher in protected areas than in non-protected areas along the East African coast (McClanahan and Arthur 2001).

In conclusion, this study shows that during the 2007 bleaching season, corals in the shallower lagoons of Kanamai and Vipingo were more resistant to bleaching stress than corals in the deeper lagoons of MMP and Nyali, probably due to a history of higher light and temperature extremes and variation at the shallower sites. Whether this acclimatization is due to short-term phenotypic changes or due to a longer-term process of adaptation through natural selection is a question that begs further research. *Pocillopora* was the genus most susceptible to mortality and *Porites* displayed high resistance to mortality. Among all these patterns of bleaching susceptibility, it is not clear whether the acclimatization is host-based or symbiont-based, and monitoring of the genetic identity of the zooxanthellae in corals at these sites could help in answering this question. Finally, bleached corals in MMP suffered lower levels of mortality than in Nyali, maybe associated with the possibility that rates of herbivory are higher in MMP due to its protected status. While herbivory is often related to resilience of reefs and the influence of herbivores assisting recovery by coral recruits, this result suggests herbivory may also mediate interactions between algae and bleached corals, and assist in the survival of bleached corals.

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Reef resilience and change 1998-2007, Alphonse Atoll, Seychelles

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Abstract. Alphonse Atoll, southern Seychelles (7°01'S; 52°44'E), a small (11.28 km^2) atoll with minimal anthropogenic influences, was severely impacted by the Indian Ocean warming event of 1997-1998. Quantitative measurements of benthic cover commenced at this location at the peak of the warming (April 1998). Underwater videography surveys of fixed transect lines were made 2001-2007, providing quantitative information on the changing benthic cover. Immediately post-bleaching, the scleractinian community was dominated by few genera, which rapidly re-colonised bare surfaces, including dead coral skeletons. Lagoon corals and many shallow water corals, presumably acclimatized to warmer water conditions, were resilient to the 1998 thermal stress and may have seeded local coral recovery. In December 2006, wave fields associated with the passage of Cyclone Bondo resulted in transport of *Porites* colonies and large blocks of reef framework onto reef-flats, loss of post-bleaching coral recruits and increased macro algal cover on the fore-reef slope. This location provides one of the most detailed long-term (> 9 yrs) records of post-bleaching reef dynamics in the Western Indian Ocean. It demonstrates i) the interaction between, and subsequent recovery from, both physiological and physical disturbances and ii) varying resilience by atoll environment (fore-reef slope, reef crest, lagoon).

Key words: Western Indian Ocean, coral bleaching, reef recovery, cyclone impact.

Introduction

The major El Niño which started in early 1997, and lasted for 12 months until a rapid switch to La Niña conditions in mid 1998, resulted in severe coral bleaching and bleaching-related mortality world-wide (Wilkinson 2000). The Indian Ocean in particular was severely affected with 'probably the most extensive bleaching ever witnessed' occurring in the central and northern Indian Ocean during the first six months of 1998 (Wilkinson 2000:23), although there were considerable regional differences in the intensity and duration of ocean warming and related bleaching (Spencer et al. 2000; Turner 1999). Individual reefs in the Seychelles suffered between 40-90% coral mortality as a consequence of this bleaching event (Bigot et al. 2000).

From July 1997 to July 1998, mean monthly sea surface temperature for the Seychelles showed a marked divergence from the long-term trend (1961-1997) (Spencer et al. 2000). Sea surface temperature remained above 30°C for three months, until April 1998, and an anomaly of nearly 2°C was recorded during February 1998, the peak of the warming event in this region (Spencer et al. 2000). An immediate post-bleaching change in the benthic community composition of the fore-reef slope of Alphonse Atoll, southern Seychelles (Fig. 1) from 1998-2003 has been previously reported (Hagan and Spencer, 2006). Here, we report new findings from 2005 and 2007, focusing on the recovery and resilience of this reef system in terms of its scleractinian community, 9 years after a major bleaching event and following interaction with physical disturbance in the form of reef aspect-controlled wave damage.

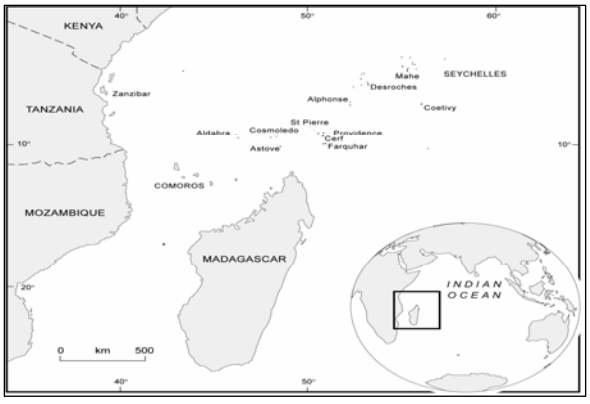

Figure 1: Location of the Seychelles, Western Indian Ocean (after Stoddart 1970). Note location of Alphonse Atoll.

Material and Methods

Alphonse Atoll (7°01'S; 52°44'E) lies at the southern extremity of the Amirantes Ridge, 415 km southeast of Mahé in the granitic Seychelles. It is a small (6 x 4 km; total area 11.28 km^2) symmetrical atoll, with peripheral reefs 640-1,900 m wide, covering an area of 4.02 km^2 (Stoddart 1984; Fig. 2). It has a 5.4 km^2 central lagoon which reaches a maximum depth of ~10m (Spencer et al. 2000).

The topography of the outer reefs at Alphonse can be separated into 3 distinct sections. Immediately seaward of the reef-flats, in less than 5 m water depth is a shallow rocky pavement characterized in places by distinct spur and groove formations. Spur and groove topography is particularly well developed on the north-west, leeward side of the atoll, where the U-shaped grooves are approximately 2 m deep. From -5 m to -15 m, a 50-150 m wide gently sloping rock surface extends down to a drop-off (Spencer et al. 2000), at -17 m to -20 m. The drop-off may either be a sheer vertical reef wall, as observed on both sides of the north-east tip of the atoll, or it may be a steep slope, as in the south-west.

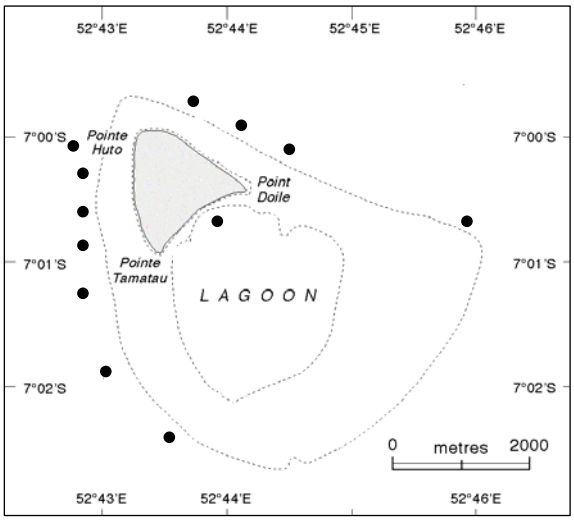

Figure 2: Location of survey sites at Alphonse Atoll; typically 1-3 depths surveyed at each site, ranging from -3.5 m to -18 m.

In 2001 and 2002, 30 permanent transect sites (29 fore-reef slope, 1 lagoon), encompassing a wide range of depths (-3.5 m to -18 m) and geographical aspects were selected. 15 m long transects were established parallel to the reef crest, marked with steel rods to aid re-location of the survey sites. Quantitative surveys were undertaken using standard video transect techniques. Video survey data was also obtained from 1998 (Hi8 format; Spencer et al. 2000) and 1999 (digital video; Teleki et al. 1999). A re-survey of all permanent transects was undertaken in 2003 and re-surveys of selected sites were carried out in 2005 and 2007.

Video data was analyzed using the AIMS 5-dot method (Osborne and Oxley 1997) to show changes in percentage cover for 7 benthic categories (sand, rubble, bare substrate, calcareous algae, Scleractinia, non-Scleractinia and macroalgae). Corals and algae were identified to genus level. Two datasets were generated; 'benthic categories data', from which percentage cover was calculated for each benthic category, and 'coral genera data'. Multivariate and univariate statistics were conducted, and dominance curves plotted, using PRIMER (Plymouth Routines In Multivariate Ecological Research) (Clarke and Warwick 2001).

At 7°S in the Western Indian Ocean, tropical cyclones are rare; frequencies for the area 0-10°S; 50-60°E are 2.1 per decade (Walsh 1984). However, in December 2006, Cyclone Bondo formed southwest of Diego Garcia and moved west across the Indian Ocean. It was a small but intense system (radius of hurricane force winds ~20 km) and category 2 strength (154-177 km h^{-1}) winds hit the Seychelles. The cyclone passed 330 km south of Alphonse Atoll, and although no terrestrial damage was observed, the associated wave field resulted in coral block detachment and transport on the northwestern side of the atoll, leaving a coral boulder debris field on the reef-flat (Plates 1 and 2). Coral blocks and dead *Porites* colonies, some measuring over 1 m in diameter, were observed in October 2007 on the reef-flat. Underwater surveys showed that these reef-flat deposits originated from depths of 7-12 m on the fore-reef slope and had been transported a horizontal distance of approximately 500 m.

Plate 1: Coral boulder debris field on northwest reef-flat, Alphonse Atoll, October 2007. Dead *Porites* colony (90 cm diameter) in foreground (photo: A Hagan).

Plate 2: Coral block measuring over 1.5 m in diameter on northwest reef-flat, Alphonse Atoll, October 2007 (photo: A Hagan).

Results

Benthic Categories Data – Temporal Community Change (1998-2007)

1998 data were only available for 5 transect lines, all of which were on the western fore-reef slope of the atoll. With such a small, localized dataset, these data cannot be assumed to be representative for the entire atoll. However, the 1998 surveys showed that live scleractinian cover ranged from 11.9-19.8% and bleached scleractinian cover values range from 8.9-19.4%. The combination of these values suggests that pre-bleaching, scleractinian cover represented between 20% and 40% of the benthos. In 1999, scleractinian cover represented between 8.8-13.7% of the benthic community, and in subsequent years, fore-reef slope scleractinian cover increased by over 5% in a 1-1.5 year period, from an average of 17.2% cover in 2001/02 to an average of 22.7% cover in 2003. However, between 2003 and 2007, there was no increase in average scleractinian cover (Table 1).

	Scleractinian Cover % ± 1 s.d.	Macroalgal Cover %
1998	14.8% ± 3.0	0.3
1999	10.3% ± 1.8	8.0
2001/02	17.5% ± 7.8	3.3
2003	22.6% ± 7.4	2.0
2005	22.2% ± 7.1	1.0
2007	21.2% ± 9.1	3.5

Table 1: Average scleractinian and macroalgal percentage cover 1998-2007 from video transect data.

Statistical tests between 1999 and 2001/02 on benthic percentage cover data showed no significant difference between these communities (ANOSIM; $P>0.05$) but there was a significant difference (ANOSIM; $P<0.01$) between 1999 and 2003.

Partitioning of these island-wide statistics shows marked differences in the time course of live coral cover changes between eastern and western survey sites. At eastern sites, a clear trend of increasing coral cover was shown 2001/02-2007 except for at the 5 m depth transect (Fig. 3). By 2007, scleractinian cover ranged between 24% and 38%. Conversely, the western sites showed a downward trend in scleractinian cover over this time period. Although scleractinian cover started off at a higher level in 2001/02 compared to eastern sites, there was a clear drop in cover in later years. By 2007, western sites displayed a maximum of 16% live scleractinian cover (Fig. 4).

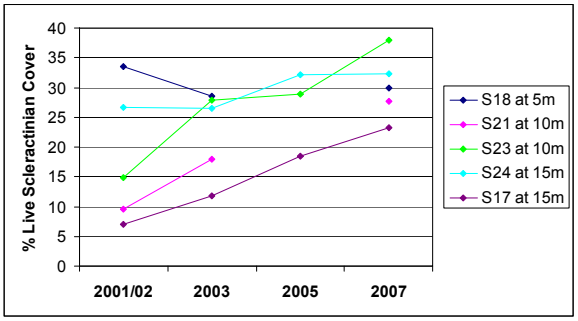

Figure 3: Scleractinian percentage cover change over time at five eastern sites. (Each line represents single transect at one depth).

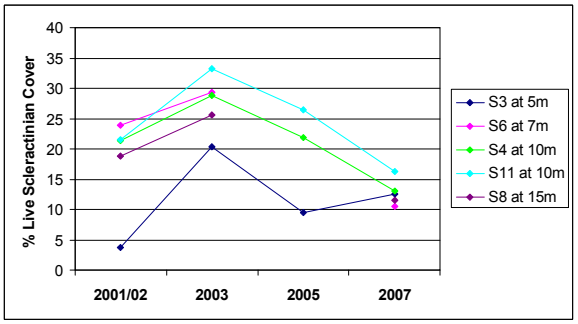

Figure 4: Scleractinian percentage cover change over time at five western sites. (Each line represents single transect at one depth).

Coral Genera Data – Temporal Community Change (1998-2007)

k-dominance curves (Fig. 5) for each of the survey years show the change in the scleractinian community composition 1998-2007. Curves for 2001/02 and 2003 cross those of 1998 and 1999, so it is inappropriate to comment on the comparison between all four curves (Lambshead et al. 1983). However, the curve for 1999 lies below that of 1998, indicating increased biodiversity in the 1999 coral genera dataset compared to the 1998 dataset. The curve of 2001/02 lies below that of 2003, indicating that the coral community of 2001/02 was more diverse than that of 2003. There appears to have been a small increase in diversity between 2003 and 2005 but this decreased again by 2007.

Coral genera diversity has decreased over time, with the lowest diversity (i.e. the highest *k*-dominance curve) being seen in 2007 (Fig. 5). The point at which the curves cross the y-axis becomes increasingly higher in later years, and by 2007, 60% of the scleractinian community was accounted for by just one genus.

Pielou's Evenness (J') (how evenly the individuals are distributed among different genera) was seen to increase between 2003 and 2005, but decrease between 2005 and 2007, supporting the findings of the *k*-dominance curves. The difference between mean Evenness values over the time series is statistically significant (ANOVA; $F = 3.53$, $P<0.01$).

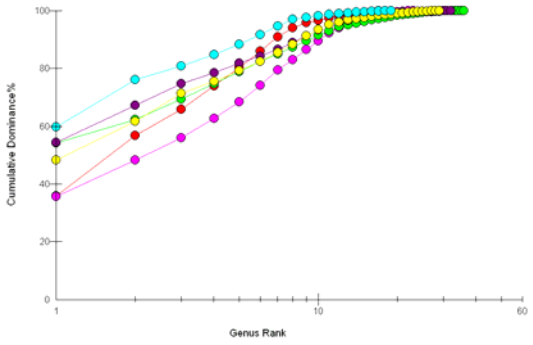

Figure 5: *k*-dominance curves for 1998 (red), 1999 (pink), 2001/02 (green), 2003 (purple), 2005 (yellow) and 2007 (turquoise) using coral genera data from video surveys.

Discussion

The 1997-98 bleaching event had a considerable impact on the community composition of the fore-reef slope of Alphonse Atoll. In 1998, at the height of the bleaching event, live scleractinian cover was reduced to an average of 14.8%, and this value had further decreased one year after the bleaching event (Table 1). By 2003, scleractinian cover had more than doubled from 1999 levels. Taking 1999 as the first year that gives a true indication of actual mortality suffered at Alphonse (1998 surveys were taken at time of on-going bleaching), this implies that a period of at least 4 years (1999-2003) was required after this major disturbance before any statistically significant recovery in scleractinian community cover could be observed.

Coral genera diversity increased between 1998 and 1999, as illustrated by the 1999 curve being lower than that of 1998 on the *k*-dominance plot (Fig. 5). However, between these two years, a decrease in live scleractinian percentage cover was calculated. The increase in biodiversity but decrease in total coral cover that was observed at Alphonse between 1998 and 1999 may imply that the bleaching event quickly removed certain genera from the 1998 dataset. However, some of these genera may have made a rapid return to the reef in 1999, increasing biodiversity levels, although their small size would not have increased the percentage cover values for that year. There was a further decrease in scleractinian diversity between 2001/02 and 2003, and again between 2005 and 2007. The *k*-dominance curves (Fig. 5) and Pielou's Evenness values indicate that in later years, there has been a reduction in diversity in the scleractinian community present and increased dominance by a few (or even just one) coral genera.

The dominant (in terms of percentage cover) scleractinian genus on the fore-reef slope of Alphonse in 2007 was *Porites*. Due to the size of many of the *Porites* colonies observed (some measured over 2 m across), it is clear that these massive, slow-growing colonies must have survived the 1998 thermal stress. In addition, lagoon corals and many shallow water corals also survived, presumably by being acclimatized to warmer water conditions (Spencer et al., 2000). The survival of these pockets of corals is likely to have improved reef resilience on the fore-reef slope at Alphonse, with these colonies acting as larval sources to re-seed degraded areas.

The second most dominant genus in 2007 was *Pocillopora*, which was widespread on all reefs at Alphonse. *Pocillopora damicornis* has been described as an opportunistic species, due to its rapid reproductive cycle and fast growth rate, enabling it to quickly occupy any newly available space (Endean and Cameron, 1990).

The granitic Seychelles islands in the north suffered over 90% coral mortality during the 1997-98 ocean warming (Wilkinson, 2000) but the southern islands were less severely affected, with an average mortality of around 60% (Spencer et al. 2000). It is suggested that this difference was due to the moderating influence of the South Equatorial Current at the southerly locations, in contrast to the heating of shallow waters, and long water residence times, on the Seychelles Plateau. As more of the corals at Alphonse survived the bleaching event compared to reefs of the granitic Seychelles islands, it can be suggested that reef resilience at Alphonse has been aided by the interaction of geographical location with regional oceanographic processes.

Another of the main factors driving reef resilience at Alphonse has been the minimal anthropogenic impact, which may have allowed more rapid natural reef regeneration than in areas with high human pressure (as has been demonstrated elsewhere; Sandin et al. 2008). Alphonse has a very small resident population (~60 people) and tourism is small-scale, with only 30 double chalets. Boat traffic at Alphonse is minimal (a maximum of 4 boats over 10

m in length were in operation at time of reef surveys) and anchoring on the reef is prohibited.

Variations in coral cover histories on reefs of differing aspect require further investigation. Cyclone Bondo caused extensive damage in a localised area on the northwest fore-reef slope, but a downward trend in live scleractinian cover was already evident in 2005 (Fig. 4). It is not known what caused this decline. One possibility is wave refraction around the atoll during the December 2004 Asian tsunami and consequent disturbance of coral cover, although general tsunami impacts in the southern Seychelles were low in littoral environments (Hagan et al., 2008).

Despite a rapid increase in macroalgal cover one year after the peak of the bleaching event, the Alphonse reefs have not succumbed to the algal 'phase shift' from a 'hard' reef community to a 'soft' reef community (Done 1999) that has been seen after reef disturbance elsewhere. An altered fore-reef slope coral community appears to exist at Alphonse 9 years after the ocean warming related mortality. Large skeletons (1-3 m diameter) of *Porites* spp., *Lobophyllia* spp., and *Symphyllia* spp. have been colonized by *Pocillopora* spp. and to a lesser extent *Acropora* spp., suggesting that the Alphonse fore-reef slope may be in an interim phase of development, through which the coral community must pass before regaining high levels of coral coverage and diversity.

Alphonse provides one of the most detailed long-term (>9 yrs) records of post-bleaching reef dynamics in the Western Indian Ocean and demonstrates not only the general interaction between physiological and physical disturbances (where human impacts can be disregarded) but also the complexities of response by atoll environment (fore-reef slope, reef crest, lagoon) and reef aspect. Continued monitoring may shed more light on the nature of these controls on reef resilience and recovery.

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Responses of coral hosts and their algal symbionts to thermal heterogeneity

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Abstract The effects of high temperature on tropical corals have been studied extensively, but little is known of their response to high frequency thermal fluctuations that are common in many reef habitats. To better understand how oscillatory temperatures affect corals, *Pocillopora meandrina* and *Porites rus* from the lagoon of Moorea were used to test the effects of fluctuating temperatures on coral physiology. Corals were incubated at a stable temperature (28 ºC), or diurnally fluctuating temperatures (26 – 32 ºC) that simulate the conditions in the lagoon. Their response was assessed through dark-adapted maximum quantum yield (F_V/F_M), *Symbiodinium* density, and holobiont respiration. Following incubations, *Symbiodinium* density and F_V/F_M were depressed 25 - 42%, and 20 - 23%, respectively, for both species under fluctuating compared to the stable temperature treatment. Additionally, respiration was 55-63% lower in the fluctuating compared to the stable conditions, although this effect was only found in one of two replicate experiments. These results demonstrate that corals can be strongly affected by diurnal temperature fluctuations in lagoon habitats.

Key Words: temperature, fluctuation, physiology, *Pocillopora meandrina*, *Porites rus*

Introduction

To date, most research on the response of scleractinian corals to thermal stress has considered steady exposures to high temperatures, even though there is evidence that many reefs experience oscillatory thermal regimes with diurnal ranges as great as 9 ºC (Lee et al. 1999, Leichter et al. 2006, Craig et al. 2001). Indirect evidence suggests that such variability can have important effects, for instance, as shown by the association between the variance in temperature and the severity of coral bleaching (McClanahan et al. 2007). The lack of attention to the effects of fluctuating temperatures on corals has important implications, as most efforts to predict the ecological consequences of coral bleaching, as well as the limits of thermal tolerance for corals (e.g., Hoegh-Guldberg 1999), are based on a tacit assumption that temperature is constant on a scale of days to weeks.

In order to understand the full range of responses of corals to thermal stress, studies are required that evaluate the impacts of fluctuating temperature, in addition to the more frequently studied effects of constant temperature regimes. The purpose of this study was to examine the effects of thermal fluctuations that are ecologically relevant for the lagoon of Moorea (i.e., a 6 ºC change within 24 h) on coral physiology. Specifically, we tested the hypothesis that rapid fluctuations in temperature have strong effects on the physiology of the cnidarian host and the *Symbiodinium* symbionts of two species of coral.

Material and Methods

To characterize the thermal regime in the lagoon of Moorea, seawater temperature at ≈3 m depth was measured using loggers (0.02 ºC resolution and 0.0017 Hz sampling frequency) between 2005 and 2006 (Fig. 1a). These temperature records revealed diurnal fluctuation with daytime heating and nighttime cooling (Fig. 1b). To test the hypothesis that such fluctuations affect coral physiology, two consecutive experiments were completed using common lagoon corals -- *Pocillopora meandrina* and *Porites rus* -- in April (trial 1) and May (trial 2) of 2006. Manipulations took place near the end of the Austral summer, which corresponds to a period of higher seawater temperature (Fig. 1a). Corals were collected from 2-3 m depth, and prepared as nubbins. The nubbins were allowed to recover from the collection process for 18-24 h in flowing seawater, and then were placed in the treatment tanks 3 d before the experiments began to allow the corals to acclimate to the ambient conditions at 28 ºC.

Experimental manipulations were used to create a comparison of diurnal thermal fluctuation, and a steady temperature (≈28.6 ºC, which served as the control and simulated mean ambient lagoon conditions during April and May 2006). The fluctuating treatment repeatedly exposed corals to

≈26ºC and ≈32ºC by transferring the nubbins daily from a tank at ≈26 ºC to a tank at ≈32 ºC at 07:00, and back to the ≈26 ºC tank at 19:00, which mimicked extreme diurnal temperature fluctuations in the lagoon. A procedural control was created by moving additional nubbins in and out of a tank held at the ambient temperature (≈28.6 ºC). Nubbins remained submerged in a beaker of seawater during all transfers, and care was taken not to touch the tissue.

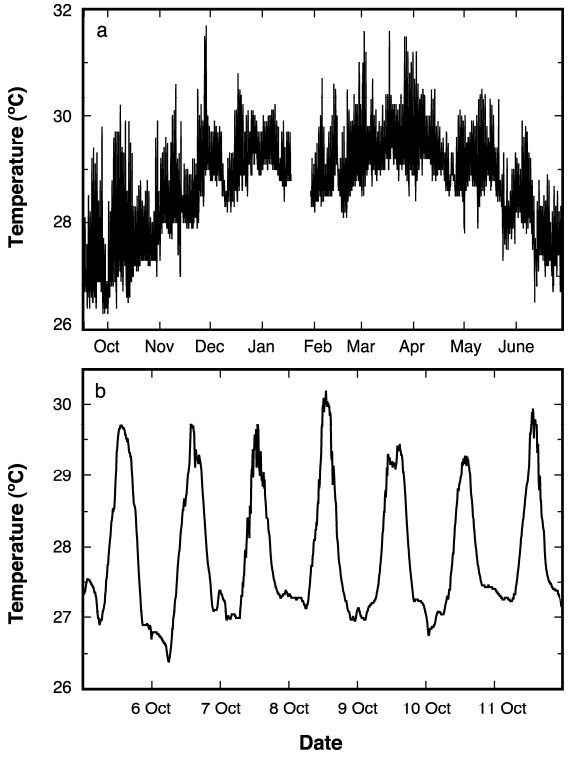

Figure 1. Representative record of seawater temperature in the lagoon (≈3 m depth, 10 min sampling interval) of Moorea from September 2005 – June 2006. (a) The 10-month record shows seasonal variation as well as high frequency changes occurring on a scale of hours-days; the ticks on the abscissa correspond to the first day of each month. (b) Seawater temperature from a 7 day period of strong diurnal fluctuation (~3.5 ºC) during October 2005. The temperature maxima correspond to midday solar warming, and the minima occur between 19:30 – 07:30; the ticks on the abscissa correspond to 0:00 hours.

The manipulations took place in an outdoor microcosm that housed 135 L tanks, each with a separate chiller, heater, and pump, and filled with seawater that was refreshed daily (~20% d^{-1}). To simulate light levels at the collection depth, the tanks were shaded to ≈1,000 µmol photons m^{-2} s^{-1} at noon. Light levels were recorded daily inside each tank, and the temperature was measured every 10 min using loggers (HOBO® and StowAway®, accuracy ±0.2 ºC). Each experiment began at 12:00 on day 1 and ended at 19:00 on day 8, and measurements began immediately following.

Response Variables- Samples sizes differed among the three variables, and also by treatment (Fig. 2). To examine the effect of the treatments, the response of the *Symbiodinium* was measured as maximum dark-adapted quantum yield of PSII (F_V/F_M) and their population density. The performance of the holobiont was measured as dark respiration of the intact coral, which reflects the combined effects of the cnidarian host and the algal symbiont.

F_V/F_M was recorded using a Diving-PAM (Walz, GmbH), and all measurements were made on the final night of the experiment, following 2.5 h of darkness. To determine F_V/F_M, each coral was removed from its treatment tank in a darkened beaker filled with seawater (500 mL), and a single measurement was made using the 5.5 mm fiber-optic probe of the Diving-PAM. A single measurement was used per nubbin to reduce the effect of the measuring light on subsequent F_V/F_M measurements (i.e. subsequent measurements result in significantly increased F_M, $t=2.67$, $df=166$, $p<0.05$). To minimize variation in F_V/F_M due to location of the probe, measurements were taken ~2 cm below the branch tips.

To quantify the *Symbiodinium*, the skeleton was removed from the fixed tissue (5% formalin) by decalcification (10% HCl). The tissue layer resulting from decalcification was homogenized using an ultrasonic dismembrator (Fisher 15-338-550) fitted with a 3.2 mm diameter probe (Fisher 15-33867). The homogenate was suspended in freshwater, and six replicate counts of *Symbiodinium* were completed using a hemocytometer. An aliquot of the slurry was dried at 60 ºC to determine biomass, and the *Symbiodinium* population density was expressed in units of cells mg^{-1}.

Dark respiration was measured as O_2 consumption within a PVC chamber (0.89 L), which was attached to a submersible pump in a closed circuit. As estimated by photographing hydrated brine shrimp eggs, flow speed inside the chamber was 23.1 ± 1.0 cm s^{-1} (mean ± SE, n=15), which approximated the mean flow speeds in the lagoon during the experimental period (R. Carpenter, pers. comm.). A single coral nubbin was placed in the seawater-filled darkened chamber, and the sealed system immersed in a 135 L tank to regulate temperature. The reduction in O_2 concentration due to respiration was measured at ambient temperature (28.2 ± 0.1 ºC [n = 24] in trial 1 and 27.8 ± 0.1 ºC [n = 26] in trial 2) over 25 min using a fiber optic probe (FOXY-R, Ocean Optics). All measurements were completed between 80 and 100% O_2 saturation to avoid O_2-dependent effects on respiration, and the rates of O_2 consumption were corrected for control values obtained by measuring O_2 consumption in a seawater-filled chamber, with no coral. Respiration rates were normalized to surface

area of the coral (obtained by aluminum foil) and reported in units of μmol O_2 cm^{-2} min^{-1}.

Statistical Analysis- The experiment was designed to be analyzed with a mixed-model nested ANOVA in which repeated experiments were used to strengthen the interpretation of treatment effects, but this design was precluded by a trial effect driven by weather-related differences in light and temperature. Due to significant differences in light regime and temperature between trials (data not shown), data were analyzed separately by trial using two-way Model I ANOVAs in which treatment and species were fixed effects, and F_V/F_M, *Symbiodinium* density, and dark respiration were dependent variables. The random placement of nubbins in each tank every day removed potential biases attributed to position effects. Data were assessed for normality and homoscedasticity using a graphical analysis of residuals, and were transformed where necessary (log transformations for respiration).

Results

Light levels within the tanks were significantly higher in trial 2 compared to trial 1 (data not shown), and corals were exposed to a slightly greater diurnal range of temperatures (i.e., a lower minimum and higher maximum) in trial 1 (5.9 ºC) than in trial 2 (5.1 ºC).

Maximum Quantum Yield (F_V/F_M)- For *Pocillopora meandrina, F_V/F_M* over both trials ranged from 0.67-0.76 in the control treatments, and 0.50-0.69 in fluctuating temperature treatments. For *Porites rus*, F_V/F_M in both trials ranged from 0.62-0.71 and 0.51-0.67 in control and fluctuating temperature treatments, respectively. F_V/F_M was significantly affected by treatment ($F_{1,55}$ =162.79, $p < 0.001$) and species ($F_{1,55}$ = 1.178, $p < 0.01$) in trial 1, but there was no interactions of the main effects ($p > 0.05$). A similar outcome of lesser magnitude was observed in trial 2, with F_V/F_M affected significantly by treatment ($F_{1,54}$ = 20.20, $p < 0.001$) and species ($F_{1,54}$ = 39.10, $p < 0.001$), but not the interaction between the two ($p > 0.05$) (Fig. 2a and 2b). These decreases of F_V/F_M corresponded in trial 1 to a reduction in the fluctuating treatment of 23% for *P. meandrina*, and 20% *P. rus*, when compared to the control treatment. The same pattern was present in trial 2, but the reductions of F_V/F_M in the fluctuating treatment were smaller (4% and 3% for *P. meandrina* and *P. rus*, respectively).

Symbiodinium Density- At the conclusion of the two experiments, most of the corals in both of the treatments were slightly pale in color (relative to freshly collected corals). The *Symbiodinium* density changed significantly for both species after exposure to the fluctuating temperatures in trial 1 ($F_{1,26}$ = 5.64, $p < 0.03$), but not in trial 2 ($p > 0.05$) (Fig. 2c and 2d). Mean *Symbiodinium* densities were reduced ~42% in *Porites rus* and ~25% in *Pocillopora meandrina* in the fluctuating treatments of trial 1. Neither the species, nor the interaction of species and treatment, were significant for either trial ($p > 0.05$).

Respiration- Mean dark respiration ranged from 0.017 – 0.046 μmol O_2 cm^{-2} min^{-1} for *Pocillopora meandrina,* and from 0.012 – 0.032 μmol O_2 cm^{-2} min^{-1} for *Porites rus* (Fig. 2e and 2f). Respiration rate was reduced significantly in the fluctuating treatment in trial 1 ($F_{1,9}$ = 13.751, $p < 0.01$) for both *P. meandrina* and *P. rus*, and differed significantly between species ($F_{1,9}$ = 4.955, $p = 0.05$). There was no significant treatment x species interaction for trial 1, or for any factor or interaction in trial 2. The reduction in respiration due to the fluctuating treatment in trial 1 corresponded to a 63% and 55% decrease for *P. meandrina* and *P. rus*, respectively, when compared to the control treatment. In trial 1, the respiration of *P. rus* was ~32% lower than *P. meandrina* at ambient temperature, and ~43% lower in the fluctuating treatment, but in trial 2, the respiration rates were statistically identical in the two corals.

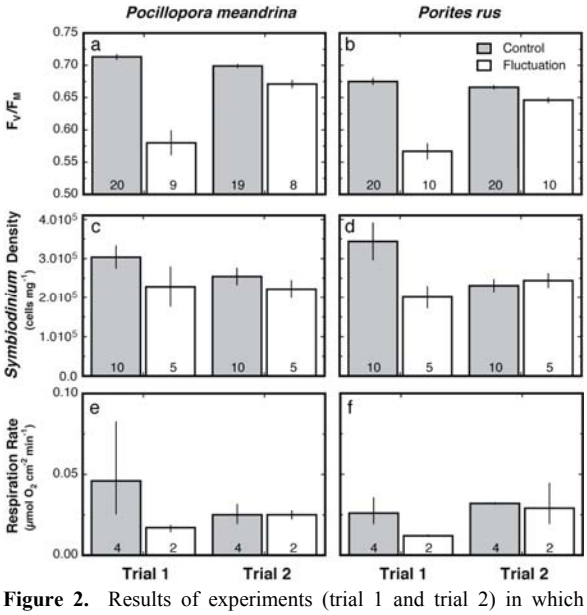

Figure 2. Results of experiments (trial 1 and trial 2) in which corals were exposed to a steady temperature of 28 ºC (control) and a fluctuating treatment consisting of 12 h exposures to ~26 and 32 ºC; (a,b) maximum dark-adapted quantum yield of PSII (F_V/F_M), (c,d) *Symbiodinium* density, and (e,f) dark respiration. Results are displayed as mean ± SE for F_V/F_M and *Symbiodinium* density, and as mean ± SD for back-transformed values for respiration; sample sizes shown within each bar.

Discussion

On many tropical reefs, corals can experience rapid changes in water temperature of a sizable magnitude within minutes to seconds (Leichter et al. 2006, Craig et al. 2001). This study presents some of the first evidence that such temperature oscillations have significant effects on corals, with the direction of response for the measured variables suggesting that the outcome is detrimental to the coral. *Symbiodinium* population density and their function (F_V/F_M), as well as the respiration of the holobiont, all were lower as a result of exposure to fluctuating temperatures, compared with corals exposed to steady temperatures. If corals respond differently not only to a consistent exposure to high temperatures, but also to the thermal oscillations, quantification of their response to fluctuations in temperature will be critical to accurately predict the response of coral communities to the changes in seawater temperature that are forecast for the next century (IPCC 2007).

In the present study, exposure of corals to fluctuating temperatures resulted in a lower efficiency of photophysiology of the algal symbionts, relative to those maintained under control conditions. The magnitude of the decrease of F_V/F_M in *Pocillopora meandrina* and *Porites rus* is likely indicative of chronic irreversible photodamage to PSII of the algal symbionts (Brown et al. 2000, Fitt et al. 2001), and the evidence for this conclusion is twofold. First, there was no sign of the depressed F_V/F_M values returning to the initial levels throughout the experiment (data not shown), as would be expected with reversible photodamage (Fitt et al. 2001). Second, the loss of photosynthetic capacity (F_V/F_M) is juxtaposed with the loss of *Symbiodinium* from the host tissue (Fitt et al. 2001), which is usually interpreted as the terminal phase of coral bleaching, and occurs in association with damage to the symbiont through oxidative stress (Lesser 1997, Fitt et al. 2001). Unfortunately, one limitation of the current study was an inability to compare the effects of the fluctuating temperature treatment and a steady exposure to each of the extreme temperatures, which is necessary to determine whether oscillatory temperatures elicit a response that is the sum of the effects of the component temperatures, or a response of a different nature.

Fluctuating temperatures reduced the F_V/F_M of *Pocillopora damicornis* and *Porites rus* by 19% and 16%, respectively. This reduction is comparable to the decrease in F_V/F_M of *Acropora palifera* and *Pocillopora damicornis* (~8 - 25%) during a natural thermal bleaching event at Heron Island in March 1998, which followed a 2-3 d period over which the seawater temperature was unusually variable (~26-34 ºC within 24h) (Jones et al. 2000). The reductions in *Symbiodinium* density as a result of fluctuating temperatures in the present study (~25 - 42%) fall within the range of losses reported during natural bleaching events (Brown et al. 1995, Jones et al. 2000, Edmunds et al. 2003). Along with clear treatment effects, there was also a significant difference between F_V/F_M for the two species. Determining why these species differ was beyond the scope of this study -- although it is intriguing to speculate that they harbor different taxa of *Symbiodinium* (Rowan 2004) -- but importantly, the absence of a treatment x species interaction for F_V/F_M indicates that *P. rus* and *Pocillopora meandrina* responded to the treatments in similar ways.

In the present study, the fluctuating temperature treatment resulted in decreased respiration compared to the control conditions for both species, at least in one of the two trials. While within the range of values reported for congeners (Edmunds and Davies 1986, Rex et al. 1995), respiration rates characteristically increase with rising temperature. A likely explanation for the lower respiration rate in the fluctuating treatment in the first trial is that it reflected the consequences of a decrease in quantity of photosynthetically fixed carbon translocated to the animal, which is thought to stimulate the metabolism of the host (Szmant and Gassman 1990), either due to a decrease in *Symbiodinium* function (as assessed by F_V/F_M), or a reduction in their population density (Szmant and Gassman 1990, Castillo and Helmuth 2005). In this situation, lower rates of respiration are likely to reflect a negative effect of temperature.

A second explanation for the decline in respiration rates in the first trial, is that it reflects a beneficial response to fluctuating temperatures (Barshis et al. 2008), and therefore could be described as acclimatization. In this situation, lower rates of respiration are arguably a positive response to temperature. However, it is challenging to interpret the implications of changes in metabolic rates, particularly in a mutualistic symbiosis where the holobiont respiration reflects the sum of the parts. Clearly, a more detailed analysis of the causes of the change in respiration rates as a result of fluctuating temperatures in the present study (as well as why it was absent in the second trial) are necessary.

In the present study, the differences between trials in the response of both corals to fluctuating temperature were unexpected given the considerable effort expended to replicate the treatment conditions. A reason for the differences in response to the fluctuating treatment between trials may be temporal variation in environmental history of the experimental corals (i.e., differences in light and temperature prior to collection). While corals were collected from the same location and depth for both trials, the additional

time (11 d) spent at local environmental conditions by the corals used in trial 2 may have had an effect on their ability to respond to extreme fluctuations. As noted previously the second trial was completed following the peak temperatures and therefore it is possible the differences in response in trial 2 may be a result of lower stress on the corals, due to the associated decrease in temperature in the field.

A greater magnitude (~16% greater) of diurnal temperature variation occurred in trial 1 compared to trial 2, and most interestingly was associated with a stronger response to the fluctuations in host and symbiont, at significantly lower light levels. This finding suggests that thermal fluctuation may act in a threshold fashion with regards to the magnitude of the fluctuations. Above this threshold, the effects of large temperature fluctuation at reduced light levels are more severe than the effects below this threshold, where small temperature fluctuation may confer protection against the effects of higher light levels, which are generally thought to accentuate thermal bleaching (Brown 1997, Hoegh-Guldberg 1999).

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Tolerance, refuge and recovery of coral communities to thermal bleaching: evidence from reefs of the Seychelles

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Abstract: Long term viability of coral communities is dictated by their ability to withstand environmental change. Three "mechanisms" exist by which reefs may survive stressful conditions: (1) physiological tolerance, (2) via environmental refuge, and (3) repopulation and growth (recovery) once ambient conditions return. During the 1998 El Niño event, sea surface temperatures (SSTs) around the Seychelles persisted above 32°C and more than 75% of all reefs bleached. Since then, SSTs have not exceeded 30-31°C allowing reefs to recover. Analyses of the size frequency distribution of species-specific colonies in the Seychelles indicated that certain species survived the 1998 event (termed type II corals) whilst other species (termed type I corals) did not but have recruited into the systems post 1998. Experiments confirmed type I and II coral species exhibited alternative physiological characteristics that likely determined the post 1998 community. Turbid lagoons containing large colonies of type I species were identified within an oceanic atoll (Desroches) suggesting that these species survived 1998 by recruiting into what have been previously considered sub-optimal habitats and were likely buffered against environmental stress. Consequently, several mechanisms are in active operation that appears to afford Seychelles' reefs some long term resistance to extreme periods of environmental change.

Key words: Seychelles, bleaching, photosynthesis, coral community structure, refuge

Introduction: Aquatic environments are subjected to environmental change, the periodicity of which occurs across a continuum from the short term, (physiological acclimation) to the longer evolutionary term (genetic adaptation). Coral reefs are no exception; in fact the near surface, shallow water, habitat of most reefs accentuates the scale of variability with which corals are exposed to life sustaining resources, primarily light, temperature and nutrition. As such, corals are adapted to living in a highly variable world; however, the Earth is currently experiencing a rate of unprecedented environmental (climate) change that vastly exceeds the rate of evolution and thus places stress on corals' pre-adaptations to environmental variability.

Typically, corals have three "mechanisms" to survive stressful conditions: (1) physiological *tolerance* until conditions return to a nominal 'steady state', which may require resources to be directed from growth and reproduction to cellular repair and maintenance; (2) *refuge* in environments that are not experiencing the same degree of stress; and (3) population *recovery* by new recruitment. Importantly, coral community structure is directly related to the extent of trophic complexity (Graham et al. 2006). Therefore, the relative activity of these mechanisms will determine future coral community composition and productivity and in turn the entire reef ecosystem. The fundamental ecological unit is the species whilst within coral systems reef building corals are the key ecosystems architects; as such, it is crucial that we understand how coral species respond to stress and how linked ecological systems will be affected. As such, it is imperative that we understand the type and extent of "mechanism" selected for in response to environmental variability by key reef building corals.

Coral reefs of the Seychelles are well recognized to have been heavily impacted by the 1998 El Niño event: sea surface temperatures (SSTs) around the Seychelles persisted above 32°C for several weeks and more than 75% (upto 95% in some cases) of all reefs bleached (Goreau et al. 2000, Spencer et al. 2000). Whilst elevated temperature is believed to be the primary factor that induces bleaching, light is also recognized as a factor that can modulate the rate and extent with which the elevated temperature impacts coral functioning (e.g. Smith et al. 2005; Brown & Dunne 2008). Bleaching describes the depigmentation of corals and is thought to occur primarily from loss of, or photosynthetic down-regulation by, the zooxanthellae (Smith et al. 2005). The coral host cannot survive for long periods without a sustained

energetic contribution by the symbiotic algae and the host coral eventually dies. Consequently, bleaching events provide an important indicator of reef response to relatively large, sometimes irregular changes in climate. ENSO events can thus be used as a 'natural' experiment to examine species/community responses to climate driven environmental stress: since 1998 records indicate that SSTs of the Seychelles have not exceeded 30-31°C potentially allowing reefs to recover from repeat exposure (e.g. see NOAA data for Mahé: www.coralreefwatch.noaa.gov/satellite). The limited data for the Seychelles suggests that in the first 5 years following the El Niño event, % live coral cover for Seychelles' islands increased from ca. 3 to 15 % and from ca. 3 to 6% for granitic and calcareous reefs, respectively (Payet, Bijou & Adam in Linden et al. 2005), and so is in an active state of recovery.

Both the immense negative impact of the 1998 El Niño event and subsequent apparent recovery of Seychelles' reefs afford an important natural system to examine how coral reefs respond to environmental change; in particular, how communities recover once growth conditions re-establish towards 'steady state' (Graham et al. 2006). We tested the hypothesis that "species existing as large colonies were least susceptible to stressful environmental conditions" by addressing 3 primary objectives: (1) Examine the size frequency distribution of colonies within a region that was very heavily impacted by the El Niño event and determine which species survived using size as a proxy for age; (2) Identify key physiological characteristics of species deemed to have survived 1998 versus those absent from the system pre 1998 (but present during 2006-7); and (3) Determine if susceptible species survived the 1998 ENSO event through refuge in light-limited environments.

Materials and methods: Coral community structure was examined at two islands in the Seychelles in August 2006 and 2007: (1) the granitic island of Silhouette (off the North East coast of Mahé) and (2) the coral atoll of Desroches (ca. 240 km off the south-west coast of Mahé). 30m continual line intercept transects were used to estimate species specific % cover and 20 m^2 quadrats (at the start of each transect) used to determine the size frequency distribution of hard corals (n = 1800 at all sites and transects). Fragments of key species (*Acropora formosa*, *A. horrida*, *Diploastrea heliopora*, *Favia speciosa*, *Fungia sp*., *Pocillopora eydouxi*, *P. verrucosa*, *Porites lutea*, and *P. nigrescens*) were taken from 5-8m for physiological measurements in the laboratory; only large (older) colonies were examined.

Respirometry— Each fragment was placed within a custom build respirometer; changes in O_2 were logged continuously using an *Optode* (Aanderaa

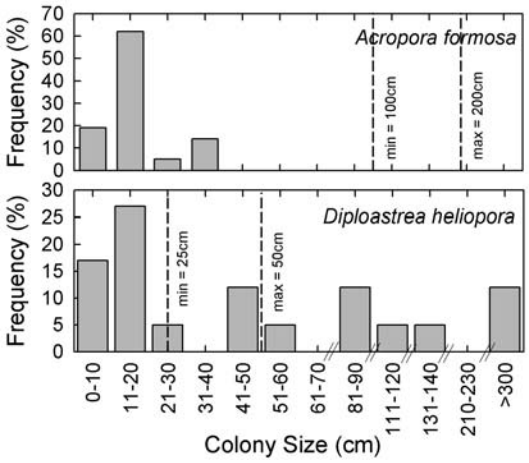

Figure 1. Size (cm)-frequency (%, of all colonies measured) distributions of key coral species from Silhouette high light environment). Examples are shown here for *A. formosa* (type I species n = 45) and *D. heliopora* (type II species, n = 60). Colonies of all other species (n = 32) identified were measured and could be classified as either type I or II corals. Dashed lined indicate size attained by 1998 based on maximum and minimum reported growth rates (see main text).

Inc., Denmark). Temperature was also logged with the *Optode* but did not increase by > 0.25 °C above ambient for any incubation: Initially, O_2 consumption was measured in the dark. A gradient of increasing light between ca. 10 and 1000 µmol photons m^{-2} s^{-1} was subsequently delivered to examine the light response of photosynthesis (P-E). Respiration measurements were again repeated at the end of the P-E run. Each light step (and respiration) was delivered for 15-20 mins. The light intensity was measured within the respirometer upon the surface of the coral using a PAR sensor after each experiment. Daily *in situ* photosynthesis and respiration rates were approximated by applying the P-E characteristics and respiration rates to measurements of the diel light climate from a HOBO (Tempcon Inc., USA) logger at the sampling site. Rates were normalized to coral surface area, as determined using the foil technique.

Thermal tolerance experiments— All fragments were placed in a community tank gradually increased over 3 hours from ambient (ca. 26ºC) to elevated (32ºC) temperature. Photosynthetic physiology was monitored prior to experimentation and then every 12 hours at the elevated temperature using diving PAM fluorometry (following Hennige et al. 2008). Changes of the maximum fluorescence yield (F_m, instrument units) and PSII photochemical efficiency (F_v/F_m, dimensionless) were used as course proxies for symbiont biomass and photosynthetic competency in response to elevated temperature. Both temperature and O_2 saturation were monitored continuously using the *Optode*. Water was changed every ca. 12 hours to minimise the reduction in O_2 saturation (never <

90%), in particular, as corals began to discolor or slough tissue from the host and into the tank.

Results: We will consider each objective in turn from data collected at Silhouette (objective 1 and 2) and Desroches (Objective 3).

Objective 1— Size frequency data were treated so as to estimate whether corals had survived the 1998 bleaching event: coral size expected for the 1998 growth year was estimated from the product of growth rate (cm y^{-1}) and number of years since 1998 (9 for Silhouette). In this way, fast growing species would of course be expected to be much larger than slower growing species assuming that 'cropping', by physical or biological processes are negligible. Growth rates used were the maximum and minimum recorded growth rates for clear waters (see Crabbe & Smith 2005). As such, the predicted sizes for 1998 were highly conservative given known inherent variability of coral growth across species, size class and environment. These predicted sizes (max-min) for 1998 are plotted as the thick vertical broken line in Fig. 2. Size frequency distributions that fall to the left of this line are indicate of colonies that have recruited post 1998; in contrast, those to the right of this line are indicative of colonies that have recruited pre-1998 and so survived the 1998 El Niño bleaching event. Of the key species examined, the acroporid and pocilloporid colonies all appeared to be post 1998 recruits (Fig. 1) whilst some species (*D. heliopora*, *F. speciosa*, *P. lutea*) exhibited colonies that were larger than would be predicted for 1998 (Fig. 1), with some *D. heliopora* and *P. lutea* colonies greater than 5m (data not shown). Corals within these two groups will subsequently be referred to as type I (recruited post 1998) and type II (survived 1998) corals, respectively.

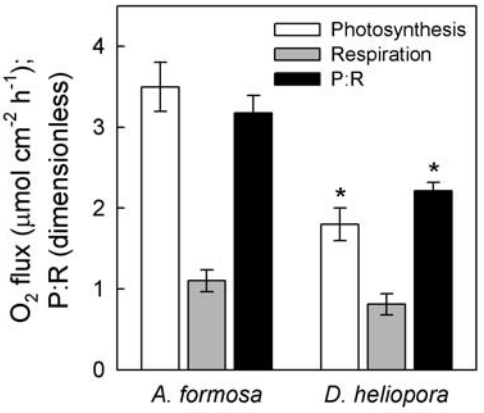

Figure 2. Rates of O_2 production (photosynthesis, P) and consumption (respiration, R), mmol O_2 cm^{-2} h^{-1}), and rates of P expressed relative to R (P:R, dimensionless). Examples are shown here for *A. formosa* and *D. heliopora* (mean ± SE, n = 3). All other species examined followed patterns the same as for *A. Formosa* (*A. horrida, P. eydouxi, P. verrucosa*, referred to as 'type I' corals) or *D. heliopora* (*F. speciosa*, *P. lutea*, referred to as 'type II' corals) (data not shown). Daily rates have been adjusted to hourly rates here. Asterisks represent significant differences between the two species for a t-test performed for each variable (i.e. P, R or P:R).

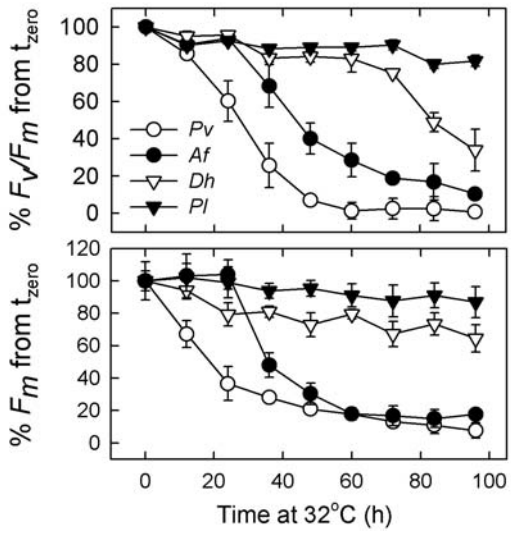

Figure 3. Mean percentage (%) ± standard error (n=3) change in photosynthetic parameters F_v/F_m (the maximum PSII photochemical efficiency) and F_m (the maximum fluorescence yield), measured by PAM fluorometry, with time of exposure of corals to elevated temperature of 32ºC (hours). Percentage change is expressed relative to values at time zero (t_{zero}). Examples are shown here for type I corals *P. verrucosa* and *A. formosa* and type II corals *D. heliopora* and *P. lutea*. All other species followed these type specific patterns (data not shown).

Objective 2— Both respirometry and PAM fluorometry demonstrated that type I and II coral species exhibited very different physiological characteristics. Rates of photosynthesis were higher by a factor of ca. 1.5-2.0 for type I than for type II corals (Fig. 2). Rates of respiration were similar across all species tested. Together these data yield photosynthesis to respiration ratios (P:R) that were highest for the type I corals (Fig. 2). PAM fluorometry was used to test for the response of type I and II corals to thermally induced stress (Fig. 3). The % change in maximum PSII photochemical efficiency (F_v/F_m) relative to time zero was greatest and occurred most rapidly for type I corals. Typically, F_v/F_m decreased by ca. 80-100% of the initial value after 40 to 60 hours at 32ºC (Fig. 3). In contrast, type II corals only exhibited a decrease of F_v/F_m by 40-20% of the initial value after 100 hours at 32ºC. Importantly, these responses by type I and II corals represent very different bleaching processes. For type I corals the decrease in F_v/F_m was the result of tissue sloughing off of the skeleton. The decrease in F_v/F_m

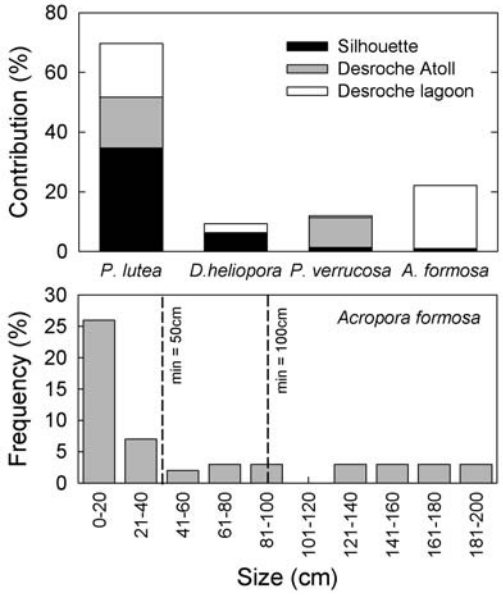

Figure 4. Relative contribution (%) of the four key coral species to the hard live coral community at each site: Silhouette (n=15), Desroches atoll (n=15) and lagoon (n=25). Lower panel is size frequency of *A. Formosa* colonies measured in the turbid lagoon (n = 53).

actually represents an increase in contamination to the minimum fluorescence yield from the skeleton (not shown) and so is not a physiological response from the zooxanthellae *per se*. This is representative of an ***acute*** bleaching response that appears to primarily target the host. Thus, the decline in symbiont biomass (i.e. the maximum fluorescence yield, F_m) is directly from a loss of coral host tissue containing the symbiont and occurs as abruptly as the change in F_v/F_m. In contrast, type II corals exhibited a gradual decline in F_m where the symbionts downregulated photosynthetic activity (Fig. 3). Here, host tissue remains in tact on the host skeleton and represents a ***chronic*** symbiont-targeted bleaching response.

Objective 3— Live coral cover estimated from all transect surveys at Silhouette and Desroches (outer high-light atoll and inner low-light lagoon) was 18.1 ± 1.1, 16.3 ± 0.8 and 7.0 ± 0.46 (mean % ± standard error), respectively. Of this cover, the relative contribution of key type I and II coral species was highly site specific. Coral abundance at Silhouette's granitic reef was dominated by *P. lutea* and *D. heliopora* but at the carbonaceous reefs of Desroches atoll by *P. lutea*; however, pocilloporids were also abundant. Importantly, the turbid waters of Desroches lagoon within the atoll were dominated by large colony sizes of the type I coral *A. formosa* and to a much lesser extent *P. lutea* (Fig. 4). Size frequency distribution of the *A. formosa* demonstrated that these lagoons contained colonies that certainly survived the 1998 El niño event. Growth rates used here were the maximum and minimum recorded growth rates for turbid waters (Crabbe & Smith 2005).

Discussion: Different species of coral appear to fall into groups that have either been susceptible to the 1998 bleaching event and only recently recruited (termed here as type I corals) or survived the 1998 bleaching event (termed type II corals). Combined with specific type I and II physiological responses, the community data provide clear evidence that several mechanisms shape the response of coral reefs to environmental variability.

Resilience— Of the key coral species tested here, acroporids and pocilloporids (our type I corals) are most susceptible to catastrophic ***acute*** thermal bleaching, a finding supported by other observations from the western Indian Ocean (McClanahan et al. 2007). Type I species are fast growing with large and successful reproductive output as opposed type II corals, which appear to reach large sizes despite slower growth rates: a clear sign that these species are resilient to periodic large changes in growth environment. Type I corals also appear to be more reliant on autotrophy as opposed to heterotrophy to sustain their metabolic needs for growth and cellular maintenance. Whilst this is obviously beneficial under 'optimal' reef building high-light environments this strategy is at a cost during extreme changes in temperature. Firstly, potential symbiont (autotrophic)-based mechanisms associated with bleaching, such as reactive oxygen production (Smith et al. 2005) will be greater where autotrophy per unit biomass of coral tissue is highest. Secondly, lower heterotrophy yields less potential to sustain metabolic demands for tissue re-growth and repair once autotrophy becomes compromised (Grottoli et al. 2006).

Alternative patterns of photosynthetic response from the elevated temperature experiment were observed for type I and II corals (Fig. 3) implying that at least two different forms of bleaching are evident. Likely, the two phenomena observed here both represent a complex composite of processes, which together can be used to describe discoloration of corals. Presumably, these phenomena may also operate in concert. Whether these phenomena are mutually exclusive in nature is debatable, in particular since our experiments here are obviously acute temperature exposure treatments, which are not necessarily representative of elevated temperature trends associated with El Niño warm water events; however, the alternative response to elevated temperature by the two coral types is consistent with the community data suggesting that the different 'bleaching' responses may inevitably prescribe the relative sensitivity to environmental change.

Refuge— Acroporids were highly sensitive to elevated temperatures yet remained the dominant coral genus in the turbid lagoons of Desroches. Here colonies were relatively large despite the lower growth rates that would be expected for the relatively low light environment (see Crabbe & Smith 2005). Temperature induced bleaching is moderated by light availability and so the observation would imply that these turbid waters offer refuge. Analogous findings have been reported for Alphonse Atoll (Iluz et al. 2008) where seagrass-based DOM release has reduced UV penetration and enabled 'type I' species to flourish. However, understanding the dominance by *Acropora* at Desroches is not so clear. It is possible that *Acropora* may have substantial potential to upregulate heterotrophy; however, this suggestion would assume that the elevated turbidity is from particulates that offer some organic enrichment to justify the energy invested in turning to heterotrophy. Alternatively, the low light environment may select against species with a lower P:R, i.e. those that are more heterotrophic, if the organic quality of particulates in the atoll was low. Unfortunately, the answer is not clear from the data available. In particular, the confounding nature of the type II coral *P. lutea* within the lagoon and of the type I coral *P. verrucosa* within the atoll cannot be explained.

Recovery— Our data suggest that Seychelles' reefs are in a stage of recovery. Percentage cover data are higher than those reported for 2004 (Linden et al. 2005). Such recovery is driven by physiological investment in growth and recruitment post 1998. Long term persistence of type I corals is driven by investment in high growth and fecundity; in contrast type II corals persist through investing into processes that regulate maintenance and physiological recovery (*tolerance*). Recruitment is a function of connectivity between reef units that act as refuge zones versus impacted zones. Whilst it is difficult to prescribe the relative contribution of recruitment of type I versus II corals, the small colony size of the fast growing acroporids and pocilloporids under 'optimal' growth conditions indicates that substantial recruitment must be accounted for by these less resilient species; furthermore, that some source for recruits must exist and be connected to the islands studied here. High recruitment was previously reported for the relatively remote Seychelles island of Aldabra (Stobart et al. 2005) suggesting that connectivity may not necessarily be a function of closely spaced reef units.

In summary, we have observed that Seychelles reefs are clearly susceptible to environmental stress beyond the thresholds for optimal growth but possess species that confer some level of tolerance. These reefs are still undergoing a phase of recovery towards pre-1998 conditions, as indicated by the relatively recent recruitment of type I corals (Fig. 1). Whether a 'true' pre-1998 community structure can ever return will of course depend on the subsequent frequency with which extreme environmental perturbations further act to 'crop' the more susceptible species and whether apparent refuges remain connected to other suitable growth environments. These refuges are often deemed to be sub-optimal growth environment for corals; however, our results demonstrate that these environments must have high conservation value.

Acknowledgements
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What is the future for extensive areas of reef impacted by fish blasting and coral bleaching and now dominated by soft corals? A case study from Malaysia.

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Abstract. Reefs in the Tun Sakaran Marine Park, Sabah, have been damaged by fish blasting and also by coral bleaching and crown-of-thorns starfish. Coral communities comprising a mixture of growth forms and including a greater proportion of massive species are recovering better than those with a high proportion of laminar and foliaceous species. An example is at Kapikan Reef, a clear-water site where there were extensive stands of laminar *Montipora* on the shallow fore reef. Collapse of these colonies created rubble that has typically become colonised by soft corals, particularly *Xenia* spp, *Cespitularia* spp (Family Xeniidae) and *Clavularia* (Family Clavularidae). Fields of soft corals have been created, covering extensive areas and acting as a barrier to recruitment of hard corals. Lack of shelter, structural complexity and food in these areas is having a significant impact on fish populations. 71% of fish at the soft-coral-dominated site fell into the 0-5cm length category, and no fish over 15cm were recorded. At the control site, only 35% fell into the 0-5cm category, and individuals up to 35cm were recorded. Currently there are no signs of recovery to a hard-coral-dominated community on the Kapikan reef, and this has implications for biodiversity, reef growth and productivity.

Key words. phase shift, xeniid soft corals, resilience

Indroduction

Globally, coral reefs are in a state of decline, with those in South-East Asia showing some of the most serious losses and degradation ((Wilkinson, 2004). Problems include over-exploitation of reef resources, destructive fishing methods, pollution, disease, coastal development and coral mortality caused by ocean warming. One outcome of these impacts and disturbances can be loss and/or replacement of reef-building corals with other organisms. Reef-building corals are an essential component of healthy reefs, but there are an increasing number of reports of phase shifts from an ecological system where hard corals are dominant to those where non-reef-building organisms such as algae or soft-bodied cnidarians are dominant (Done 1992, Hughes, 1994, Chen and Dai 2004). A better understanding of the factors affecting resistance (ability to withstand impacts) and resilience (ability to recover) can help managers and other stakeholders decide on appropriate strategies and interventions that may help to stem further coral reef decline and promote recovery. This paper describes the response of reefs in Sabah, Malaysia to disturbances that have led to a phase shift from hard corals to soft corals, and have had a secondary impact on fish populations and biodiversity.

Materials and Methods

The Tun Sakaran Marine Park is situated off the south-east coast of Sabah, East Malaysia (Fig. 1). It was gazetted in 2004 and covers an area of 350 km^2. The reefs have been monitored since 1998 using rapid assessment techniques and quantitative surveys based on Reef Check methodology as described in Hodgson et al. 2006. A Reef Check monitoring site was first established on Kapikan Reef in 1998, and was re-surveyed in 1999, 2000, 2004 and 2007. Fish censuses were carried out on the same reef in May 2008 at sites dominated by soft corals, with a control site on the adjacent Mantabuan reef. For the fish censuses, counts were made along 10 belt transects, each 5m wide x 20m in length. Each 20m transect was split into 5-m sections and all specimens were recorded. Fish were identified to species where possible, or to family, and were assigned visually to size classes.

Results

The Kapikan reef is approximately 3.5km in length and has a gently-sloping profile. The shallow fore reef (1 – 5m depth) is up to 100m wide and has a very poorly developed spur and groove formation manifested as low undulations. The reef rim is not

M

well defined, but curves gently, with a slightly increased degree of slope below about 10m depth.

Figure 1. Location of study site: Tun Sakaran Marine Park. Kapikan reef (K) Mantabuan reef (M)

Hard and soft coral cover

Preliminary surveys along this reef in 1998 revealed areas of intact coral, with laminar and leafy *Montipora* and staghorn *Acropora* common, but with many other genera and growth forms present. Xeniid soft corals were widespread and common, sometimes forming patches many metres in diameter. In addition to the healthy stands of live coral, there were places where corals had been broken, probably as a result of fish blasting that is widespread in the area. In 1998, significant bleaching of hard corals due to an increase in sea surface temperature was noted on this reef, with as many as 30% of the colonies affected.

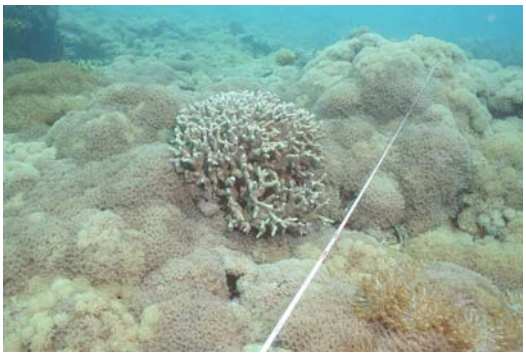


Figure 2. Kapikan reef 2007, heavily colonised by *Clavularia* and *Xenia* at a depth of 5m

Rapid assessments made along this reef annually since 1998 have revealed a gradual increase in the proportion of soft corals, and an expansion of the patches to form extensive fields (Fig. 2). The soft corals involved are *Xenia* spp, *Cespitularia* spp (Family Xeniidae) and *Clavularia* (Family Clavularidae). *Braireum* and *Efflatounaria* are also present, forming smaller patches. The soft corals have successfully colonised rubble, much of which on this reef consists of plates up to 15cm in diameter. Larger pieces of old coral and the tops and sides of scattered limestone blocks created by the death of massive corals on this reef have also mainly become colonized by the same assemblage.

Results from the Reef Check monitoring site established on this reef in 1998 show an upward trend in cover by soft corals on both the 4m and 9m depth transect. Soft corals were already well established on the shallow transect in 1998 (43% ±SE 2.68) and by 2007 cover had increased to 75.63% ±SE 2.43 (Fig. 3). On the 9m depth transect soft coral cover rose from 0.6% ±SE 0.25 in 1998 to 38.75% ±SE 2.25 in 2007.

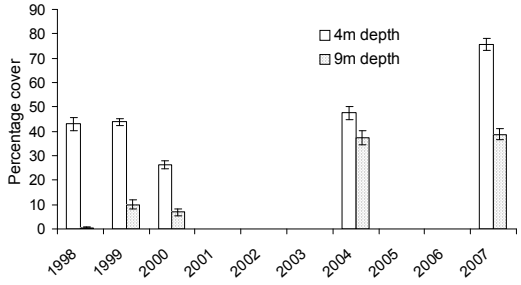
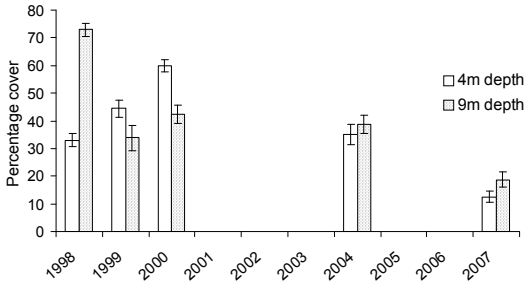


Figure 3. Percentage cover by soft corals at the Kapikan monitoring site

Live hard coral has fallen over the same period (Fig. 4). Hard coral cover on this reef could be declining for a number of reasons. Destructive fishing, crown-of-thorns starfish and bleaching have all been recorded and may be acting separately or synnergistically. During the first Reef Check survey in 1998 bleaching was in evidence, with an estimated 30% of colonies bleached at 9m and 10% at 4m



depth.

Figure 4. Percentage cover by hard corals at the Kapikan monitoring site

Damaged reefs in other parts of the Park show different responses. Rapid assessments over the same period, together with detailed information from 10 other monitoring sites, show that in many places where shallow-water coral assemblages dominated by *Acropora* and other branching corals have suffered damage, they have been reduced to smaller fragments of rubble. Unlike the larger rubble pieces on the Kapikan reef, these areas have not been colonized either by hard or soft corals.

In habitats where massive hard corals have died, there have been phase shifts to alcyoniids such as *Sinularia*, *Litophyton* and *Sarcophyton*, but this is not as comprehensive as the xeniid-*Clavularia* invasion seen on the outer reefs. Xeniids and *Clavularia* also occur on the inner reefs, but form discrete clumps rather than extensive fields.

Fish censuses

Fish counts were carried out on a typical section of the Kapikan reef dominated by xeniids and *Clavularia* and on a section of the adjacent Mantabuan reef where invasion by xeniids and *Clavularia* had not occurred. Both sites are subjected to similar levels of fishing.

Mean soft coral cover at the fish census site on Kapikan was 66.6% (± 7.94) in comparison with only 2.04% (± 0.65) at Mantabuan.. Conversely, mean hard coral cover was 3.6% (±0.99) at Kapikan and 46.62% (± 5.02) at Mantabuan. Both census sites had similar amounts of rubble (around 20%), but the Mantabuan reef had a greater proportion of rock (16.75% ±2.54) than Kapikan (2.85% ±0.85).

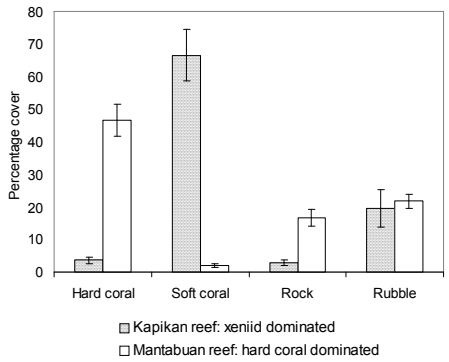


Figure 5. Main benthic components at the fish census sites

A total of 683 individual fish were recorded in the ten 5m x 20m transects on the xeniid-*Clavularia* dominated reef. Observations at the micro-habitat scale revealed that these fish were associated with rubble patches or clustered around the small number of surviving hard corals. Maximum fish length was 15cm, but the majority of fish (71%) were in the 0-5cm size category. The majority of these specimens were small labrid and pomacentrid species and mean density in the 11-15cm size class (the largest represented) was only 3/100m^2 (Fig. 6).

On the Mantabuan reef a total of 1,452 fish were recorded, of which only 35% were in the 0-5cm size category. 21% of individuals were in the 11-15cm size category (in comparison with 4.5% on the Kapikan reef) and the maximum size of fish recorded was 35cm. Representatives from all trophic levels and a wide variety of families were present on the Mantabuan reef, including lutjanids, pomacanthids, scarids, serranids and chaetodontids as well as labrids and pomacentrids. Mean density in the 11-15cm size class was 10 times higher (30/100m^2) than on the xeniid-dominated reef (Fig. 6).

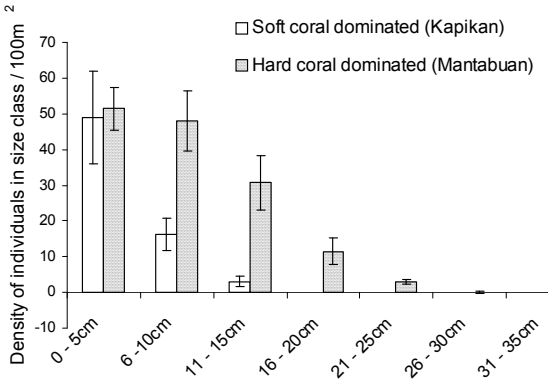


Figure 6. Density of fish recorded on the Kapikan (soft coral dominated) and Mantabuan (hard coral dominated) reefs according to size class.

Discussion

The reefs described in this paper are situated within the Tun Sakaran Marine Park, which was gazetted in 2004 and is currently the largest MPA in Malaysia. The Park lies within the coral triangle and the reefs are of value for their high biodiversity, natural resources and recreational interest. However, like many others in the region, they have been damaged and degraded, particularly by destructive and over-fishing, coral bleaching and predation by crown-of-thorns starfish.

Although the relative contribution to reef decline from each of the different impacts/activities has not been quantified, one of the outcomes at some sites has been a phase shift from hard corals to a predominately xeniid-*Clavularia* assemblage. This

particular community change has developed only on the gently-sloping eastern-facing outer reefs where the water is clearest, and where laminar hard corals (primarily *Montipora*) had been common. When these corals collapsed they produced relatively large pieces of rubble that acted as suitable 'settlement plates' for xeniids and their allies. The tops and sides of scattered limestone blocks created by the death of massive corals on these outer reefs have also mainly become colonized by the same assemblage.

Xenia, Cespitularia, *Clavularia* and related forms are well know for their ability to colonise vacant space on reef surfaces, and can spread rapidly by cloning where conditions are suitable (Fabricius and Alderslade, 2001). It appears from this study that the combination of clear water, gentle slope and a disturbed but sufficiently stable substratum on the Kapikan outer reef has given xeniids a competitive advantage over other organisms. Their strategy of larval brooding and effective asexual propagation enables fast clonal growth (Fabricius and Aldersade, 2001) and their success is further enhanced by their ability to produce allelopathic substances that chemically inhibit growth and survival of other organisms (Sammarco et al, 1983, Fabricius and Alderslade, 2001).

Damaged reefs in other parts of the Park show different responses. In many places, shallow-water coral assemblages dominated by *Acropora* have been reduced to smaller fragments of rubble that have not, as yet, been colonized either by hard or soft corals. These reefs experience similar tidal current and wave exposure to the xeniid-*Clavularia* dominated reefs, but the fragments are probably too unstable to allow colonisation. Where stable limestone blocks remain, alcoynid soft corals belonging to the genera *Sinularia* and *Sarcophyton* are typical colonists. Xeniids occur, but do not form the extensive fields seen on the outer reefs. Similar habitat preferences have been described for the Great Barrier Reef, where alyconiid 'invasions' are associated with disturbed nearshore reefs (Fabricius, 1998) while xeniids invade outer shelf sites (at > 3m depth). In the latter cases, however, the dominant genus was *Efflatournaria*, and the patches were reported to be not larger than tens to hundreds of square metres.

In Komodo National Park (Indonesia) large fields of the soft coral *Xenia* often grow on top of rubble created as a result of fish blasting (Fox et al. 2003). The genera *Sarcophyton*, *Nepthya* and *Clavularia* are also present in these *Xenia* fields.

Phase shifts to cnidarians other than alcyoniids have been reported from a number of locations, and mainly involve coralliomorphs (Kuguru et al. 2004; Tkachenko et al. 2007; Work et al. 2008). A shift to domination by the sea anemone *Condylactis* has been reported from southern Taiwan (Chen and Dai, 2004) following pressure on reefs over two decades from overfishing, coastline development and tourism.

The fields of xeniids now colonizing large sections of the Kapikan outer reef have created an impenetrable blanket that provides few hiding places or feeding grounds for fish. The fish that are present are mainly small species of labrid and pomacentrid that are associated with isolated patches of rubble or small rocks and corals within the xeniid-*Clavularia* fields. This represents a significant reduction in value of the reef for biodiversity, local fisheries and tourism.

The fact that few large fish were recorded even at the hard coral dominated sites could be related to the relatively short transect lines (20m). However, numerous fish surveys conducted throughout the Park using 50m transects confirm that large fish are very scarce. Mean density of reef fish such as groupers and rock cod, snappers, emperors and sweetlips at 12 monitoring sites recorded annually since 1998 has typically been less than one individual per family per100m^2, and at several sites has been zero (Wood, 2008). The reefs in the Park have been heavily fished for decades using hook and line, nets, traps and explosives, so it is not surprising that fish density is low and sizes are small.

The ability of the reef to recover from this phase shift is unknown, but it appears that currently the xeniids are successfully competing with the remaining live hard corals in addition to spreading actively over damaged areas. Thus they are not only forming a physical blanket preventing settlement and recruitment of hard corals, but they are also killing those that remain.

Experimental work has shown that reefs exhibiting a phase shift to algal dominance can recover in situations where herbivorous fish are abundant (Bellwood et al 2006; Hughes et al 2007). However, a parallel scenario is unlikely to exist for xeniids because they have few natural enemies. The only reason why they might die back is from senescence or from mortality as a result of bleaching or physical disturbance (e.g. storms).

Xeniids are known to be susceptible to bleaching (Fabricius and Alderslade 2001) but hard corals are also affected and so a warm water event would most likely cause die-back of both soft and hard coral communities. It is possible that storms could result in the rubble and/or soft corals being dislodged, although wave action is not an important modifying factor at this location. The east coast of Sabah is outside the hurricane zone, and severe storms are relatively infrequent. If the underlying rubble was again 'laid bare' as a result of storms or die-back of the xeniids due to bleaching there is no

certainty that hard corals would prevail over soft corals when re-colonisation occurred.

It is difficult to predict what, if any, management interventions might be effective in enhancing the ability of the reefs to revert to hard coral domination. It has been shown that phase shifts to algal domination can be prevented or dramatically slowed down by the grazing of herbivorous fish. Thus a management 'insurance policy' to prevent such phase shifts from occurring is to manage fish populations so that over-fishing of large herbivores such as scarids and labrids does not occur. However, in the absence of effective soft coral 'grazers' it is difficult to know what options exist for managing xeniids and their relatives.

Experimental work on soft coral fields in the Komodo National Park (Fox et al 2003) showed that small hard corals were present beneath the soft coral canopy and 'fared much better' if the soft coral was removed. In addition, hard corals recruited to the cleared rubble, resulting in an increase from an average of 2.94 colonies per m^2 when the soft coral canopy was first cleared to an average of 7.15 colonies per m^2 after 6 months. In another experiment, Fox et al showed that *Acropora yongei* nubbins attached to PVC pipes and elevated 5-10cm above the soft coral canopy survived better than nubbins that were at the same level as the canopy. Survival was 70% after 6 months for the elevated nubbins in comparison with 30% for the nubbins in the canopy. The researchers concluded that soft corals inhibited recruitment, growth and survival of hard coral over the short term but suggested they could facilitate hard coral recovery in the long term. This is because the soft corals stabilise the rubble, and if gaps open up, hard corals may successfully settle in these more stable patches. It would be useful to carry out similar trials at the study site in Sabah to see if clearance of patches within the *Xenia* fields leads to an increase in hard coral cover.

In conclusion, human activities and impacts have changed the ecological character of the Kapikan reef from a reef-building to a non-reef-building state, and this altered ecology currently shows no sign of reverting back. Continued studies will determine whether or not the xeniid-*Clavularia* assemblage is a climax community, or whether this set of dominant organisms will be replaced by another or even revert to hard corals. Recovery might occur if the soft corals die back or are torn away by storms, and it is possible that clearance of patches within the *Xenia* fields could be beneficial.

It is anticipated that increased surveillance and enforcement in the area will lead to a reduction in fish blasting and that this will prevent more sections of the reef being reduced to rubble. It is vital that remnant populations of hard coral remain healthy because they will provide a source of coral larvae that could aid recovery of damaged areas.

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The maintenance of reef islands

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Abstract. Low coral reef islands are known to comprise biogenic sediments but the processes whereby these islands are nourished are poorly understood. This paper argues that the sediment system of large reef platforms can be strongly compartmentalised, at sub-kilometre scales, so that contemporary island maintenance and growth depends on organisms and processes operating within a small physiographic and ecological sector of the reef flat. Evidence is provided through a study of the origins and pathways of island-nourishing sediments on Warraber Reef, using textural, compositional and selected dating analyses of surface sediments. Results reveal that the mollusc-algae covered sand flats of the emergent, inner reef flat are the dominant contemporary source of island sediment. In contrast, deeper, more-distant and leeward reef-flat zones are functionally isolated from the island beach deposit. This is despite the order of magnitude greater productivity, more-frequent submergence, and more-energetic wave environment of the latter areas, but in-line with reef flat sediment transport directions and the proportionately-greater particle production rates of organisms living in the windward, near-island zones. The key findings of this study are summarised in a theoretical model of the source-to-sink journey of skeletal biogenic sediments in low island environments, which is applicable to reef-island management.

Key words: Reef islands, Carbonate sediment, Platform reef, Reef flat.

Introduction

By 2015 half the world's population will live in coastal zones, including on low coral reef islands. These reef-surface features are described as particularly vulnerable to changing environmental conditions such as sea level rise (Woodroffe et al. 2007). They are constructed of accumulations of biogenic sediment primarily from the surrounding reef platform (Hart and Kench 2007). Beaches form the crucial interface through which these islands are nourished, built and eroded with reef platform sediments.

Little detail is known about the relationship between the different bio-physiographic zones of reef platforms and the ultimate nature of island sinks. A number of seminal papers have demonstrated that the character of reef sediments depends on: the carbonate producing and eroding organisms present (Stearn *et al*. 1977, Scoffin *et al*. 1980, Scoffin 1987); the contributing organisms' skeletal structure (Scoffin 1992, Ginsburg *et al*. 1963, Chave 1964, Enos and Perkins 1977); and on their interaction with biological, physical and chemical processes of breakdown and transport within reef environments (Folk and Robles 1964, Maiklem 1968, Gourlay 1988).

This paper contributes to this discourse through an investigation of how spatial variations in sediment processes across large reef platforms can influence the nature of a low-island beach. Comparisons between beach and reef-flat deposits reveal functional relationships between the island sink and reef sources for Warraber Reef, an intertidal platform in central Torres Strait, Australia. The differing spatial and organism contributions to contemporary beach nourishment found are used to model the functioning of reef sediment systems and discuss their importance in understanding reef island futures.

Study Site

A large platform (11 km^2) and small, oval-shaped sand cay (1.1 km^2) form the Warraber Reef system (Fig. 1). Warraber is middle of 3 platforms called The Three Sisters, situated at the northern end of the Great Barrier Reef in central Torres Strait (10°12'S, 142°49'E). The Strait is characterized by 3.5 m spring tides, tidal currents up to 4 ms^{-1}, and a monsoonal wind and wave regime. Strong winds from the southeast (~15 ms^{-1}) dominate the dry season (March-Sept) while weaker northwesterlies (0-5 ms^{-1}) occur during the wet season.

Warraber (or Sue) Island stands 2 to 8 m above mean sea level (MSL) on the northwestern reef platform. It is thought to have developed incrementally over the last 3500 years while the reef surface (0.5 to 2.3 m above MSL) and much of its outer structure grew during the Holocene, around a Pleistocene reef core (Woodroffe et al. 2007). Rimmed by a young coral-algal rim, the platform's

contemporary reef flat has two distinct areas: the large, elevated central and eastern section with sand flats covering fossil microatolls and branching corals; and the smaller, lower, western reef flat characterized by muddy-sand flats near the beach and coral patches separated by sand channels towards the rim. The boat channel, constructed in 1991, divides the western reef flat in two. On low tides more than half of the reef flat drains fully while water ponds across deeper, western and outer-eastern, areas.

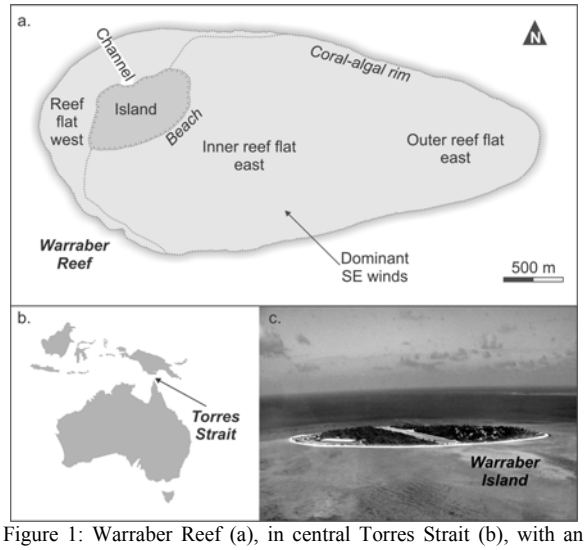

Figure 1: Warraber Reef (a), in central Torres Strait (b), with an oblique aerial photograph from the south side of the island (c)

Material and Methods

218 surface sediment samples were hand scooped along 15 beach to inner reef flat profiles and 5 transects radiating out from the island to reef rim. 3 additional samples were dredged from the boat channel at 11 m below MSL. Samples were cleaned of organic matter, and the fines were removed by wet sieving (particles <0.063 mm). The remainder was split into 15 and 70 g subsamples.

The 70g subsamples were settled through a 6.5 m long water column into a collection core, which was then separated into 4-6 visually-distinct fractions (after Kench and McLean 1996). 100 grains were grid-sampled from each fraction and their constituent origins determined using a reflected-light binocular microscope. Constituent weights were summed across all fractions to determine their % in each sample. For constituents comprising >1% of samples, Wilcoxon rank-sum tests were used to determine if variation between the beach and reef flat sample groups was significant (Zar 1999). Agglomerative, hierarchical cluster analysis was performed on the complete sample constituent data using the *S-Plus* software programme *Agnes* (Everitt 2002) and the sample constituent clusters mapped across the reef platform.

The 15 g subsamples were settled through a rapid sediment analyzer. Sample settling distributions and statistics were calculated using Middleton's (1967) negative $\log_2$ Psi parameter (ψ) and Folk's (1965) graphic formulae. Cluster analysis was performed separately on the textural data using *Agnes* and the textural cluster trends mapped across the reef flat.

Results

Table 1 shows the main constituents (≥1%) that made up the reef flat and island beach sediments. The commonest sediment constituent was mollusc (mostly gastropod), comprising 55% and 35% of beach and reef-flat samples respectively. The next commonest constituents were coralline algae (16-26%, mostly encrusting) and coral (8-13%). On the beach this was followed by *Halimeda* (7%), foraminifera (5%), and fragments of beachrock (2%) while on the reef flat, the fourth commonest constituent was foraminifera (10%), followed by *Halimeda* (8%), crustacean (4%), and vermetid mollusc (2%).

Amounts of the two main constituents, mollusc and coralline algae, were significantly different (P>0.01) between the beach and reef flat sample groups. Of the less-common constituents, beach and reef-flat sediments contained differing amounts of foraminifera, crustacean and beachrock material but significantly similar amounts of coral, *Halimeda* and vermetid mollusc. The mean sediment compositions reveal that, on average, the beach comprised more mollusc and beachrock fragments while the reef flat had more coralline algae, foraminifera and crustacean sediment than occurred on the beach (Table 1).

Table 1: Variation in the mean constituent composition of beach versus reef flat (RF) sediments (* indicates P<0.01 in Wilcoxon rank-sum variance tests). Reef flat cover and carbonate production are included for comparison, where cover represents the 24% of the reef flat occupied by carbonate producers (Hart and Kench 2007)

Organism type	Mean % sediment composition		Mean % of live cover	Mean % of carbonate
	Beach	RF	RF	RF
Mollusc (gastropod)	54.9* (47.7%)	34.8* (27.8)	3 -	4 -
Coralline algae (encrusting)	15.6* -	25.6* -	47 (46.5)	18 (16.2)
Coral	8.2	13.1	43	74
Halimeda	6.9	8.4	6	1
Foraminifera	4.8*	10.2*	1	2
Vermetid mollusc	0.6	1.5	<1	<1
Crustacean	0.6*	3.5*	<1	<1
Beachrock fragment	1.9*	0*	-	-

Comparisons between these sediment results and reef flat ecology data from Hart and Kench (2007) reveal that, on average, *Halimeda* and the minor constituents (<4%) were represented in the two sediment sinks in similar proportions to their reef flat cover and/or carbonate budget contributions (Table 1).

However, the occurrence of the other, major constituents in the sediments contrasted their parent organisms' reef flat cover and carbonate productivity. Coral, and to a lesser extent, coralline algae, appear to be under-represented as sediment constituents, while molluscs and, to a lesser extent, foraminifera appear over-represented, relative to their reef flat cover and productivity. In order to investigate explanations for these differences, surface-sediment spatial patterns were mapped based on the cluster analyses of % constituent compositions, and the textural properties.

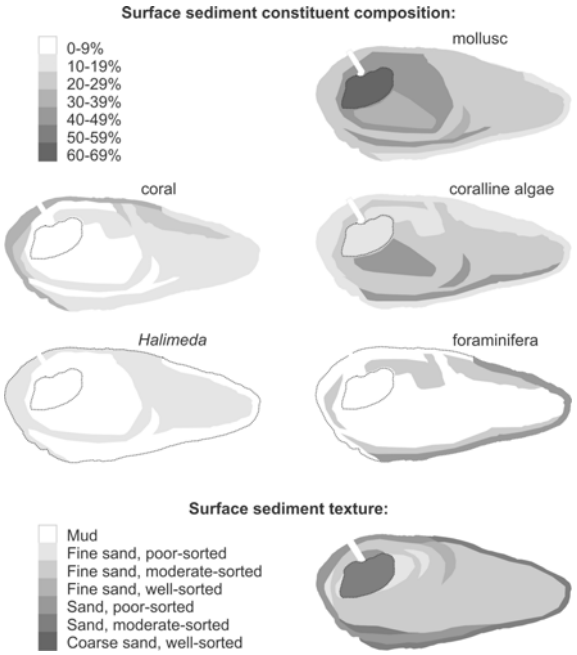

Figure 2: Trends in the concentrations of the five main sediment constituents in the beach deposit, represented by the island shape, and across the reef flat (top); and corresponding textures (bottom)

Fig. 2 shows that mollusc sediment concentrations generally increased from deeper-outer areas, towards central-emergent areas of the reef flat, and were greater in the beach deposit than anywhere on the reef flat. Almost the exact reverse pattern was evident for coral sediments. Coralline algae content decreased gradually south to north across the reef flat, and between the reef flat and beach deposits. *Halimeda* constituents were slightly more concentrated in deeper reef flat areas compared to on the beach and inner, emergent reef flat. The concentration of foraminiferal tests was high around the eastern and southern reef rim and patchy across the rest of the platform. Fig. 2 also reveals that sediment texture was coarser and better-sorted in the beach deposit and on the reef rim, relative to across most of the reef-flat surface. The boat channel sediments were dominated by poorly-sorted mud of indistinct origin.

Discussion

Two key results requiring explanation are that Warrraber beach appears to contain disproportionally high concentrations of mollusc sediment and low amounts of most other constituents.

Textural results revealed the island shore as a high-energy setting relative to much of the surrounding reef flat. Mollusc shells are ideally suited to transport to, and retention within, such a deposit as they are buoyant and transportable as well as durable compared to the majority of other reef constituents (Chave 1964, Force 1969). Further, for most molluscs on Warraber (which were small gastropods) the translation of live-parent organisms into sediment particles suitable for nourishing the beach simply entails death. This contrasts coral and encrusting coralline algae, which require the additional step of skeletal breakdown to form particles suitable for beach nourishment.

The dominance of mollusc in the beach deposit is also due to the concentration of suitable mollusc sediments on the inner reef flat surrounding the island (Fig. 2). This corresponds to a concentration of live molluscs in this area (Hart and Kench 2007). Dating of individual particles by Woodroffe et al. (2007) reveals that molluscs form the youngest fractions of Warraber's island and reef-flat deposits, aged between modern and 2700 y, supporting the idea of a link between contemporary mollusc production on the inner reef flat and the concentration of mollusc sediment in the island beach.

Like molluscs, foraminifera have tests that are highly-transportable, durable, and simply translated into sediment upon death of the parent organism. Unlike molluscs, however, they form the oldest constituents in Warraber's island and reef-flat deposits (6600-4500 y old, Woodroffe et al. 2007). This indicates that, while foraminiferal material can persist in this reef environment, there may have been a decrease in the generation of new material over time. Today most foraminifera live far from the island on the reef rim, with a few living nearshore, east of the island (Hart and Kench 2007). This live-assemblage pattern is similar to the observed foraminiferal sediment pattern (Fig. 2). Accordingly, the slightly-lower average concentration of foraminifera in the beach relative to reef-flat sediments found in this study (Table 2) is explained by the location of concentrated foraminiferal sediments either far or up-drift from the island shore (according to the dominant-southeasterly wind wave regime) as well as by the dilution of the beach constituents with the abundant mollusc material.

The proportions of *Halimeda* and coral constituents in the island beach reflect their average concentration in reef flat deposits (Table 2). *Halimeda*'s role in the

sediment budget reflects its minor role as a reef flat cover type and in carbonate productivity. This is not the case for coral, which was shown by Hart and Kench (2007) to be the second commonest live-carbonate-producer cover type and, by far, the dominant carbonate producer. Reasons as to why this extensive coral cover and carbonate production does not translate into dominant sediment contributions in either the reef-flat or island deposits include, first and foremost, that even in this emergent-platform environment, the bulk of coral carbonate produced is retained within the reef framework upon tissue death and not broken into sediment particles. Coral only grows where there is accommodation space. On Warraber, coral growth is concentrated in outer-reef-flat and reef-rim environments, where there is horizontal and vertical accommodation space respectively. Also, when coral skeletons do break down, their skeletal architecture, in combination with bioerosion processes, commonly leads to the production of a bimodal sediment population: that is, gravels which are readily worn into silts and muds (Scoffin 1987). The former are ill-suited to transport across the reef flat to the island shore, particularly from the western areas (where coral sediments are most concentrated) as this is against the dominant wave regime. The latter, fines cannot be retained in the high-energy, coarse beach deposit (Fig.2).

The path of coralline algal sediment generation is slightly different again. This carbonate producer is the most-extensive living-cover type on Warraber Reef, after the non-carbonate producing brown algae (Hart and Kench 2007). But this cover translates into to far-less carbonate per unit live cover than for coral, so that there is less coralline algal material available for potential sediment generation. Compared to its role in the carbonate budget, coralline algae makes up a slightly-greater proportion of the reef flat, and slightly-smaller proportion of the island-beach, sediment budgets. Its reef-flat sediment contributions may be explained by its consistently-high cover across the reef flat, including growth on existing sediment particles - characteristics which predispose it to key sediment contributions. The slightly lower concentration of coralline algae in the beach may, in turn, be the product of winnowing of this not-so-durable constituent (Chave 1964).

Implications

The International Coral Reef Symposium (ICRS 2008, 3) scientific outcomes overview states that recent evidence indicates tropical reef systems are “primarily connected at scales of tens of kilometers”. This comes from a biological perspective and is counter to pervious beliefs that reefs are highly interconnected at scales of thousands of kilometers. The present research suggests that low-reef islands are connected to reef ecosystems at even-more-local scales. That is, in terms of beach nourishment (and thus island-building), the sediment system of the large, emergent Warraber reef platform is highly compartmentalized in space and time. The most-important beach constituent (mollusc) is largely locally (<1 km) and recently (≤2700 y) produced while the dominant carbonate producer (coral) and live-cover types (brown algae, coralline algae, coral) on the wider reef platform contribute far less to the maintenance and development of the contemporary island shore.

Based on these findings, Fig. 3 outlines the source-to-sink journey of skeletal biogenic sediments in low island environments. This model combines carbonate and sediment-particle production stages with the morphodynamic concept of low islands as the product of reef-flat sediment transport and nodal-point deposition. It shows that reef platform deposits, including cays, are inextricably linked to their surrounding ecosystems, and that this relationship is highly compartmentalized via the complex set of biological and physical processes involved in the creation, alteration and transport of reef and reef-island sediments.

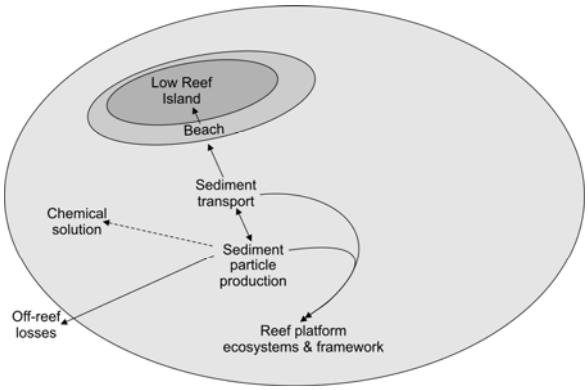

Figure 3: Theoretical schematic of a reef island sediment system, including carbonate production in surrounding platform ecosystems; its translation into sediment via producer-organism death and/or biological or mechanical erosion; and sediment transport to the beach for island nourishment and development, deposition in the reef edifice, or losses offshore and via chemical solution.

Current models indicate that reef-flat widths, elevations, current strengths and directions, and sediment volumes are all important in determining the rate and nature of island development (e.g. Gourlay 1988, Kench and Brander 2006, Barry et al. 2007). These factors are largely outside of the control of reef managers. The model developed in this study indicates that the amounts, and constituent and textural natures, and spatial distributions of sediments produced in reef flat environments today are crucial in

determining the island-beach sediment budgets and, thus, island maintenance, development or erosion. One major implication of this model is that it is necessary to understand the carbonate and sediment production systems of reefs in order to understand the future of their islands under changing environmental conditions such as those brought about by climate change, increased pollution and resource pressures. On Warraber, the reef-island sediment budget depends on production by a limited set of organisms, across a limited area of reef surface. The particular organisms and reef flat zones responsible for island maintenance will differ from reef to reef and change over time - establishing these locally can help reef managers to forecast the future of islands and empower them with information about the importance of conserving particular reef-flat ecosystems for island maintenance.

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Coral recruits to settlement plates at remote locations in the U.S. Pacific

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Abstract. Documenting the density and taxon of coral recruits to settlement plates is a widely used method of quantifying coral recruitment. Four deployments of recruitment plate arrays were made at six locations in the Northwestern Hawaiian Islands at annual or biennial intervals between 2001 and 2006. Similar arrays were deployed at Kingman Atoll, Palmyra Atoll, Baker Island, and Rose Atoll for three consecutive, 2-year periods and at Jarvis Island for one, 2-year period. Most locations with multiple deployments showed temporal variability in the density of coral recruits. In the Northwestern Hawaiian Islands, lowest average recruitment rates (8.5 and 10.4 recruits m^{-2} yr^{-1}) were found at Midway and Pearl and Hermes Atoll, respectively, and highest average rates were found at Maro Reef (265.3 recruits m^{-2} yr^{-1}). Annual rates at the five remote central Pacific locations ranged from 0.8 to 16.3 recruits m^{-2} yr^{-1}. The taxonomic composition of the recruits did not reflect the composition of adult coral communities. These results reveal the need for further study of coral reproductive and recruitment processes in these remote areas where little data have been collected.

Key words: corals, recruitment, settlement plates

Introduction

The reported decline of coral reefs (e.g., Pandolfi et al. 2005) necessitates improved understanding of their intrinsic capabilities for replenishment. The capacity of scleractinian corals to maintain or renew genetically diverse populations through sexual reproduction is a key attribute of reef resilience (West and Salm 2003). Recruitment patterns can be an important factor affecting the future coral community (Coles and Brown 2007).

Documenting the density and taxon of coral recruits to settlement plates is a widely used method of quantifying coral recruitment (Mundy 2000). Recruitment studies involving artificial substrata are typically conducted at locations readily accessible from land-based institutions, and the substrata are submerged for several months (e.g., Polachek, 1978; Fitzhardinge, 1985; Kolinski 2004). Studies from more isolated regions that are distant from supportive land bases are rare (e.g., Hughes et al., 1999).

The Northwestern Hawaiian Islands (NWHI) are a chain of small rocky islands, atolls, coral islands, and reefs that span ~1800 km over more than five degrees of latitude in the northwestern portion of the Hawaiian Archipelago (Fig. 1). Designated as Papahānaumokuākea Marine National Monument in 2006, they are uninhabited except for seasonal field camps or small maintenance crews at several locations. Given the expanse of the region, most marine research activities require support from large institutional research vessels.

Situated closer to the equator but spread across more than 5×10^6 km^2 of the tropical Pacific Ocean are five small islands/atolls (Kingman Atoll, Palmyra Atoll, Jarvis Island, Baker Island, Rose Atoll; Fig. 1) that are managed by the U.S. Fish and Wildlife Service. As in the NWHI, research is largely conducted aboard large research vessels.

No previous studies of coral recruitment have been conducted in any of these locations. A pilot study was launched in the NWHI in 2001 to examine spatial and temporal variability of the density and taxonomic composition of coral recruits at six locations by attaching settlement plates to the anchors of oceanographic instruments. Similar methods were applied to the five remote central Pacific locations beginning in 2002. This paper, which presents the results from a 5-year study in the NWHI and a 6-year study in the remote central Pacific locations, is a first look at coral recruitment in these locations.

Material and Methods

An array ("cohort") of 16 (15 cm × 16 cm) unglazed terra cotta plates was deployed at six locations in the NWHI (French Frigate Shoals, Maro Reef, Lisianski, Pearl and Hermes, Midway, and Kure) and five remote locations in the central Pacific (Fig. 1) for consecutive periods of ~1 or 2 years. At the end of each deployment period, the entire submerged array

and plates were retrieved and a new array with fresh plates was deployed on the same day.

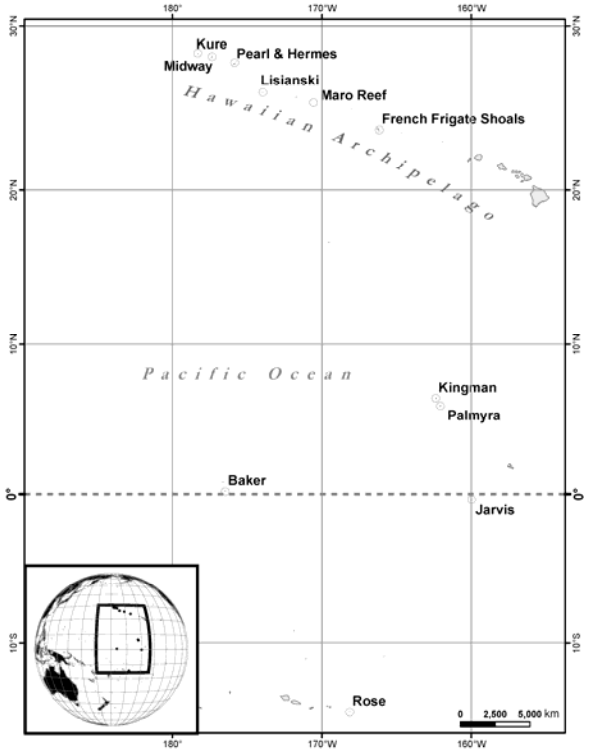

Figure 1. Deployment locations of settlement plates.

The plates were assembled in a framework of polyvinyl chloride (PVC) pipe in which grooves had been cut to hold the plates. Arrays deployed at < 10 m depth were attached with cable ties to the anchor of an oceanographic buoy. A sheltered site was selected to moor the buoy and arrays, where they might withstand the long deployments. At Baker and Jarvis Islands, there was no suitable protected shallow-water (<10 m) habitat in which to deploy a surface buoy. Instead, the PVC framework with plates was attached to an anchor in deeper water (~ 15–20 m). Of the 16 plates, 8 were placed horizontally and 8 vertically.

After retrieval, the plates were rinsed of excessive sediment and algae, frozen, and returned to Honolulu. Both sides of each plate were examined for coral recruits with a dissecting microscope. Recruits were classified in the family Pocilloporidae, Acroporidae, Poritidae, or unidentified based on skeletal criteria of Babcock et al. (2003), with the exception of the first cohort from the NWHI when this diagnostic reference was not available. Density of recruits (no. m^{-2}) was calculated based on the number of retrieved plates and the available settlement surface area of each plate. In calculating annual recruitment rates (no. m^{-2} yr^{-1}), densities were normalized to the number of days the arrays were deployed at each location.

Results

Northwestern Hawaiian Islands

Most locations showed temporal variability in the annual rates of coral recruitment (Fig. 2a). At all locations, the annual recruitment rate on plates deployed from 2004 to 2006 was substantially less than that of previous cohorts. The highest average annual recruitment rate (265.3 recruits m^{-2} yr^{-1}) was found at Maro Reef, and the lowest average annual rates (8.5 and 10.4 recruits m^{-2} yr^{-1}) were found at Midway and Pearl and Hermes Atolls, respectively.

Of the 891 recruits tallied on plates from the second, third, and fourth cohorts, 96.2% belonged to the family Pocilloporidae, 2.7% to the Acroporidae, and 1.1% to the Poritidae (Fig. 2b).

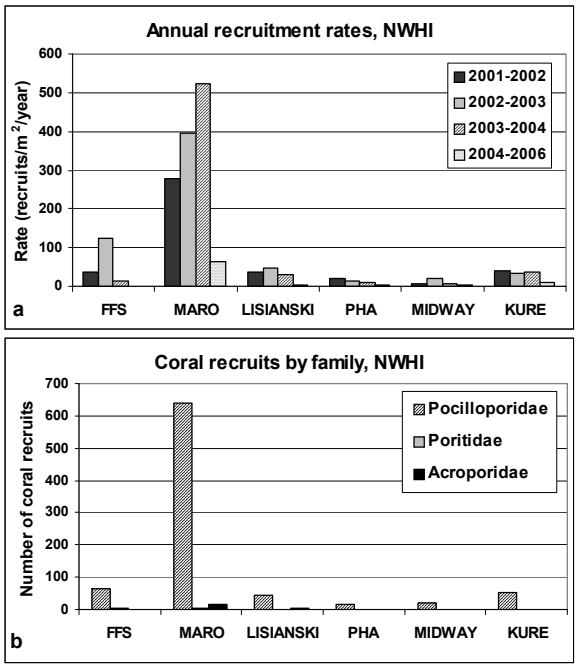

Figure 2. Coral recruitment parameters from settlement plates in the NWHI between 2001 and 2006. a. Recruitment rates (no. m^{-2} yr^{-1}). b. Taxonomic composition by family. FFS = French Frigate Shoals; PHA = Pearl and Hermes Atoll

Remote Central Pacific Locations

At Jarvis Island, the entire first cohort of plates had become detached from the anchor and was not found, presumably due to the high wave energy environment. Only 9 of the 16 plates from the second cohort were recovered; hence, a third array of plates was not deployed at Jarvis. There was temporal variability in annual recruitment rates at Kingman Atoll, Palmyra Atoll, and Baker Island (Fig. 3a). At Rose Atoll, only one coral recruit was found from all three, 2-year deployments. The highest average annual rate (8.1 ± 2.8 recruits m^{-2} yr^{-1}) was found at Kingman Atoll and the lowest average annual rate (0.3 ± 0.2 recruits m^{-2} yr^{-1}) was found at Rose Atoll. The highest average

annual rate in the remote central Pacific locations was lower than the lowest average annual rate in the NWHI, at Midway Atoll.

Of the 68 coral recruits found from all remote central Pacific locations, 25.0% belonged to the family Pocilloporidae, 20.6% to the Acroporidae, 32.4% to the Poritidae, and 22.1% could not be identified. The relative contribution of each taxon varied by location (Fig. 3b). At Kingman Atoll and Baker Island, the dominant component of the recruiting taxa also varied by cohort. At Kingman Atoll, acroporids dominated the first cohort (80% of recruits, n = 10) but poritids dominated the final cohort (90% of recruits, n = 20). At Baker Island, pocilloporids were exclusively found in the first and second cohorts (i.e., 100% of recruits, n = 7, 4, respectively) but acroporids dominated the third cohort (42% of recruits, n = 12). At both Kingman Atoll and Baker Island, settlement plates were deployed and retrieved at comparable times of the year.

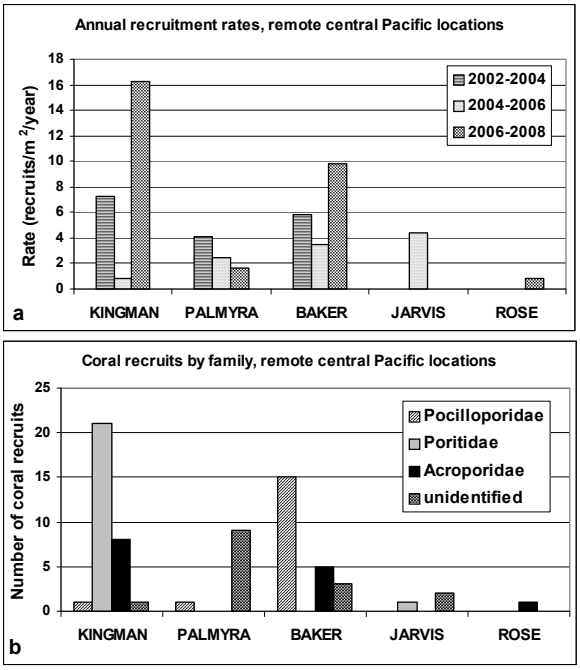

Figure 3. Coral recruitment parameters from settlement plates at remote central Pacific locations between 2002 and 2008. a. Recruitment rates (no. m^{-2} yr^{-1}) b. Taxonomic composition by family.

Discussion

Northwestern Hawaiian Islands

At each location the placement of the anchor to which the plates were attached was based on protection by neighboring reef structure from severe wave activity. Consequently, the arrays were deployed in varying geomorphological habitats with variable live coral composition of adjacent reef. These habitat variations preclude statistical comparison among locations in recruitment rates or taxonomic composition.

Kenyon (1992) determined that three species of *Acropora* are sexually mature at French Frigate Shoals from June to August, but no other studies of coral reproduction exist from the NWHI. It is reasonable to assume, however, that the mode and timing of gamete or planula release is similar to that displayed by conspecifics in the main Hawaiian Islands (MHI). With the exception of the 2003–2004 cohort, settlement plates were deployed in September and October, towards the end of the reproductive periods displayed by the suite of spawning taxa (Kolinski and Cox 2003), and likely missed the peak larval settlement period of that year. The 2003–2004 cohort, which was deployed in July and August, provided settlement substrate during more peak reproductive periods, which may account for the higher densities of that cohort at Maro Reef (Fig. 2a).

The taxonomic composition of the recruits was disharmonic with the composition of the adult coral communities in this region. The genus *Porites* dominates at all islands/atolls in the NWHI both in terms of percent cover and colony density (Kenyon et al. 2006; 2007a,b), but only ~1% of coral recruits were poritids. In contrast, pocilloporids made up >96% of the recruits although their contribution to percent cover ranges from 3.5% at Maro Reef to 33.1% at Kure Atoll (J. Kenyon, unpubl. data). Year-round release of planulae by *Pocillopora damicornis* (Kolinski and Cox 2003) may contribute to the disharmonic representation of this genus in the coral recruits. The contribution of adult acroporids to percent cover ranges from 1.5% at Laysan (Kenyon et al. 2007b) to 21.6% at Kure (J. Kenyon, unpubl. data) but only 2.7% of all the recruits were acroporids. In a 2-year study of recruitment in Kaneohe Bay, Oahu, in the MHI, Fitzhardinge (1985) observed that there was no obvious relationship between the abundance of adult colonies at a site and the number and taxonomic composition of the recruits. In a 24-year study of visible (> 0.5 cm) recruits at four sites off Kahe Point, Oahu, Coles and Brown (2007) also noted that *Pocillopora meandrina* generally had higher recruitment rates than *Porites lobata*. Nonetheless, the extreme paucity of poritid and acroporid recruits in the present study highlights the need for better understanding of coral reproductive, recruitment, and early life history processes in this region, and has important implications for recolonization by larvae should populations of these taxa be negatively impacted by natural or anthropogenic disturbances.

At all locations, annual recruitment rates calculated from the 2004–2006 cohort were lower than those of previous cohorts; this decrease was especially pronounced at Maro Reef, where recruitment declined

from 522.6 recruits m^{-2} yr^{-1} to 65.0 recruits m^{-2} yr^{-1} (Fig. 2a). Most islands/atolls in the NWHI experienced mass coral bleaching in 2004 (Kenyon and Brainard 2006), and responses to bleaching can include diminished reproductive capacity (e.g., Omori et al. 2001) and reduced recruitment (e.g., Obura 2001). However, *Pocillopora*, the major component of the recruits, was not heavily bleached at Maro Reef (Kenyon and Brainard 2006). The suite of causes behind this reduction in recruitment remains unclear.

The highest average annual recruitment rate was found at Maro Reef. The mesh of reticulate reefs adjacent to the lagoonal site where the plates were deployed may have facilitated retention of gametes and/or larvae compared to other NWHI locations where the neighboring reef structure was not as complex. Brown (2004) noted in the MHI that sites with higher recruitment rates were either in protected embayments or along coastlines with strong currents adjacent to reefs with high coral cover.

Remote Central Pacific Locations

The varying coral faunas and range of habitats in which the arrays were deployed at the five remote central Pacific locations preclude statistical comparison among locations. Each location is treated separately in the following discussion.

The arrays at Kingman Atoll were surrounded by a dense population of *Porites lutea* colonies. One hundred fifty-five coral and anemone species have been reported from Kingman Atoll (Brainard et al. 2005), but little is known of the timing of coral reproduction. Kenyon (2008) inferred that several episodes of spawning involving multiple *Acropora* species take place annually over 2 or 3 months beginning in late April at Kingman and Palmyra. Fresh settlement plates were deployed biennially before this inferred initiation of spawning and provided substrate for two annual periods of reproductive activity. Recruitment to the 2004–2006 cohort of plates was among the lowest rate documented in all remote central Pacific locations during the 6-year study (Fig. 3a), and the reasons behind this failure in recruitment are unclear.

The arrays at Palmyra Atoll were deployed in the lagoon, which experiences limited circulation and low coral abundance (J. Maragos, pers. comm.). One hundred sixty-eight coral and anemone species have been reported from Palmyra Atoll (Brainard et al. 2005), but little live coral is presently found in the lagoon. The consistently low recruitment rates (Fig. 3a) likely reflect the depauperate nature of coral communities in this portion of the lagoon.

The plates at Baker Island were deployed in a region of moderately high (~ 36%) coral cover dominated by *Acropora nobilis* (Coral Reef Ecosystem Division (CRED), unpubl. data). Eighty-eight coral and anemone species have been reported from Baker Island (Brainard et al. 2005) with assemblages dominated by *Montipora*, *Acropora*, *Pavona*, and *Pocillopora*, but no studies of coral reproduction have been conducted here. The average annual recruitment rate (6.4 ± 1.2 recruits m^2 yr^{-1}) was second only to that at Kingman Atoll.

The plates at Jarvis Island were deployed in a region of moderately high (~ 40%) coral cover dominated by *Montipora* and *Pocillopora* (CRED, unpubl. data). Forty-nine coral and anemone species have been reported at Jarvis Island (Brainard et al. 2005), with assemblages dominated by *Montipora*, *Pocillopora*, and *Pavona*, but no studies of coral reproduction have been conducted here. Recruitment rates were the highest from all remote central Pacific locations for the 2004–2006 cohort (Fig. 3a), but in the absence of data from more than one cohort, other generalizations cannot be made.

The arrays at Rose Atoll were deployed in the lagoon close to a patch reef with moderately high coral cover (~ 45%) dominated by *Favia*, *Montipora*, and *Astreopora* (CRED, unpubl. data). Observations of spawning by seven species (Itano and Buckley, 1988; Mundy and Green, 1999) off Tutuila, ~340 km distant from Rose Atoll, following the October or November full moon constitute the best available data by which to predict reproductive phenology of the 111 species of corals and anemones (J. Maragos, pers. comm.) at Rose Atoll.

All Locations

Average annual recruitment rates at all locations in the NWHI were higher than those at remote central Pacific locations, although adult coral cover is lower at all locations in the NWHI than any of the remote central Pacific locations (Kenyon et al. 2006, 2007a,b; CRED, unpubl. data). Hughes et al. (1999) also noted a lack of conformity between adult abundance and recruitment on the Great Barrier Reef. The range of substrata, depths, faunal diversity, submergence times, and metrics used to report recruitment in published scientific literature (Field et al. 2007) confounds comparisons with rates in the present study. With this caution, recruitment rates from studies in the MHI range from 0.2 recruits m^{-2} yr^{-1} (Polachek, 1978) to 1536 recruits m^{-2} yr^{-1} (Kolinski 2004). In a study of recruitment on the Great Barrier Reef at four hierarchical spatial scales, Hughes et al. (1999) determined that recruitment by broadcast-spawning corals was most variable at the largest spatial scale (sectors, each 250–500 km apart) and least variable among reefs within sectors, each 10–15 km apart. Future studies of recruitment in the NWHI, where the islands/atolls are separated by the scale of

sectors, could benefit from a similar hierarchical design, and future studies in all locations could benefit from spatial replication.

Acknowledgements

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INDIRECT EVIDENCES ON THE CONNECTIVITY OF CORAL REEFS OF THE GULF OF MEXICO AND THE MEXICAN CARIBBEAN

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Abstract. Coral reef connectivity results from the export and import of species or reproductive product between localities. Possible exchange pathways between the reef ecosystems in the country are not known; such knowledge about coral reef connectivity could contribute to its management and conservation. The connectivity between reefs of the Gulf of Mexico and Mexican Caribbean was evaluated based on patterns of similarity. Information for 48 stony coral species in 19 localities was compiled from different sources. Species richness suggests that the highest coral biodiversity is located around Cozumel on the Caribbean with 33 species. Cluster analysis based on biological similarity between localities shows that the Veracruz Reef System (VRS) is more similar to the reefs of the Mexican Caribbean than those on the Campeche Bank. Correlation (Mantel test) of biological similarity with geographical distance, days of transport by currents and environment variables, was negative and highly significant, corroborating that biological similarity decreases with increasing distances. The hypothesiss that the reefs of the VRS and the Caribbean are more similar because these areas are less affected by hurricanes is proposed. This environmental stability would lead to an accumulation of Caribbean coral species that makes VRS more similar to the Caribbean than to those reefs in the Northern Veracruz or those in the Yucatan shelf.

Key words: Connectivity, Dispersion, Coral reefs, Similarity

Introduction

Coral reefs are open systems that exchange organisms, nutrients and reproductive products, wholly or partly mediated by water flow (Sale, 2004). The density of populations occupying the reef ecosystem depends in part on reproductive effort of the species in remote areas where gametes are released, on the eficiency of mechanisms of transport in terms of larval viability and level of connectivity, and on the own reproductive capacity and recruitment of local populations (Roberts 1997). Most coastal marine species have limited adult movement, so the relative short-lived pelagic larval stages represent the primary opportunity for dispersal. Following the pattern of surface currents, some attempts have been made to try and define the transportation routes that are followed by larval stages, resulting in the recognition of general pathways of connectivity amongst the different areas where coral ecosystems occur (Cowen et al. 2006).

Connectivity in the marine environment can also be constrained by biogeographic barriers as seen in terrestrial environments, which are not easy to discern, due to the short duration of larval stages and dispersal mechanisms by currents (Thorrold 2006). One way to infer connectivity from the ecological point of view, is to determine the similarity of ecosystems from background information such as species richness, number species shared, the similarity of environment and their relationship to geographical distance. In general, those communities that are farthest from each other should be the less similar due to their lower connectivity (Nenkola and White 1999; Steinitz et al. 2006). Such relationships between the reef ecosystems of the Gulf of Mexico and Mexican Caribbean is unknown, so better knowledge should contribute to improve decision-making in management and conservation.

Material and methods

Records of species of corals of the Gulf of Mexico and Mexican Caribbean (Fig. 1) were compiled from seven different sources with comparable methodology (Table 1). Data were organized in tables according to species abundance, dominance, and Shannon-Wiener diversity (Magurran 1988).

Depending on the parameters of the community, analysis of qualitative biological similarity was carried out first with data on presence/absence by applying the Jaccard and/or Sorensen indices (Nenkola and White 1999; Steinitz et al. 2006). Similarity was determined with the Manhattan distance as a measure of affinity and the method of Ward as a strategy of agglomeration.

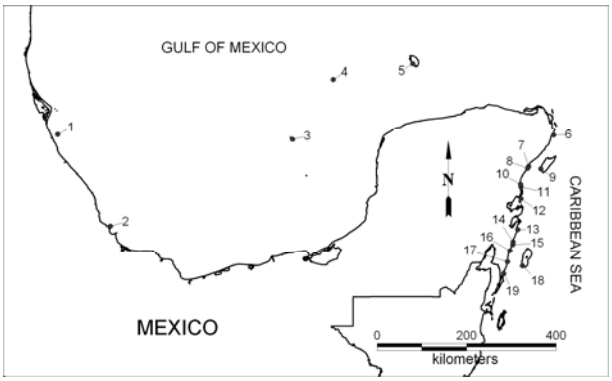

Figure 1. Corals reefs considered in the Gulf of Mexico and Mexican Caribbean: 1. Tuxpam, 2. Veracruz Reef System, 3. Triángulos, 4. Cayo Arenas, 5. Alacranes, 6. Punta Nizuc, 7. Akumal, 8. Chemuyil, 9. Cozumel, 10. Boca Paila, 11. Punta Yuyum, 12. Punta Allen, 13. Tampalam, 14. El Placer, 15. Chaguay, 16. Mahahual, 17. Xahuayxol, 18. Chinchorro Bank, 19. Xcalak.

Table 1. Coral reef localities and source of data included in this study. A = abundance data; P = presence data

Data	Locality	Source
A	Akumal	1, 3
P	Alacranes	4, 7
A	Boca Paila	1
A	Cayo Arenas	4, 7
A	Chaguay	1
A	Chemuyil	3
A	Chinchorro Bank	1, 4
A	Cozumel	3
A	El Placer	1
A	Mahahual	1, 2, 3
A	Punta Allen	1
A	Punta Nizuc	5
A	Punta Yuyum	1
A	Veracruz System	5, 6, 7
A	Tampalam	1
A	Triángulos	4, 7
P	Tuxpam	6, 7
A	Xahuayxol	1
A	Xcalak	1

Source: 1. Marks and Lang (2005); 2. Aguilar-Ontiveros (1998); 3. Borges-Souza & Chávez (2007); 4. Chávez et al. (1985); 5. Gutiérrez et al. (1993); 6. Pérez-España (2007); 7. Horta-Puga et al. (2007)

Similarity was also explored using other indices (Legendre & Legendre, 1998). Correlation of biological similarity with the geographical distance, the days of transport by currents and environmental similarity was assessed using simple and partial Mantel test (Fortin and Gurevitch 1993). Geographical distances were estimated using a geographic information system (GIS), to construct the matrix of distances and the matrix of days of larvae drifting being transported by currents (mean annual velocity, Mariano et al., 1995). A matrix of environmental similarity was calculated using mean annual environmental factors (sea surface temperature, salinity, oxygen, nitrates, phosphates and silicates) obtained from the NOAA World Ocean Atlas 2005 (http://www.nodc.noaa.gov).

Results

Coral communities had the highest species richness (S) in Mahahual, with 37 species. The highest ecological diversity (Shannon-Wiener) was found in Cozumel, with 4.05 bits/ind. Exploration of presence/absence data did not allow us to find any clear pattern defining how biological similarity declines with increasing geographic distance; for this reason, the following analysis was performed using quantitative data of relative abundances of coral species.

Classification of sites allowed to identify a pattern of grouping the reef localities. Veracruz Reef System (VRS), in the southern Gulf of Mexico, shows higher similarity with localities in the Mexican Caribbean than with localities in the Northern Gulf of Mexico or with those on the Yucatan shelf (Fig. 2)

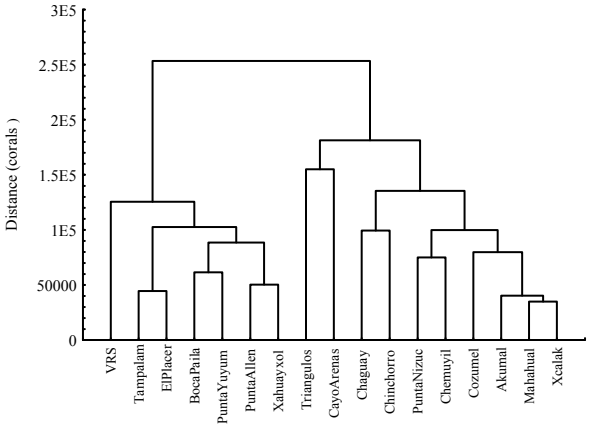

Figure 2. Cluster analysis (Manhattan distance, Ward method) for coral reef localities.

The first two Principal Components based on environmental variables explained 78.6 % of the total variance, with nitrates (0.98) and silicates (0.88) correlated positively with the first component. In the second component, the salinity showed positive correlation (0.89) whereareas sea surface temperature correlated negatively (-0.94).

The arrangement of localities in ordination space depicts a gradient of variability, with the reefs from the south and central Mexican Caribbean and the reefs from the Gulf of Mexico caracterized by low concentration of nitrates and silicates and relatively high concentration of phosphates (Fig. 3). According to the second principal component, the Mexican Caribbean localities were caracterized by warmer surface waters, but less salinity, while the Gulf of Mexico localities were more salty and waters with lower temperature (Fig. 3).

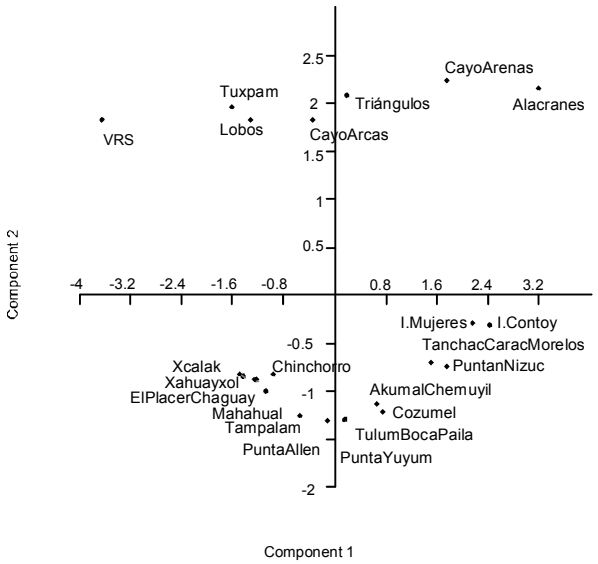

Figure 3. Principal Component analysis using components 1 and 2 as gradients of environmental variability (78.6% of total variance explained.)

A simple Mantel test was used to test the correlation of coral reef similarity with geographical distance, with the distance in days of transport as effect of current and with the environmental similarity showing negative correlations in all cases, except for biological similarity and days of transport by oceanic currents. In such statistical significant tests, the biological similarity between localities decreases as the geographical distance between them increases (Table 2).

Table 2. Simple Mantel test correlation between similarity and relevant distances matrices for coral reef localities. Correlation *r* and statistical significance (*p*) after 10,000 randomizations.

Simple Mantel test	*R*	*p*
biological similarity vs. distance (km)	-0.514	0.018*
biological similarity vs. days of transport	-0.384	0.076
biological similarity vs. environmental factors	-0.615	0.003*
distance (km) vs. days of transport	0.962	0.001*
environmental factors vs. distance (km)	0.885	0.001*
environmental factors vs. days of transport	0.751	0.001*

(*) statistical significance p<0.05

Prior to the partial Mantel test, the simple correlations between the matrices of the independent variables were obtained. All the combinations between them were positive, higher than 0.75 and highly significant (Table 1).

Table 3. Partial Mantel test for relevant matrix combination (vs.) with fixing effects from the third matrix (and) for coral reef localities. Correlation *r* and statistical significance (*p*) after 10,000 randomizations.

Partial Mantel test	*r*	*p*
biological similarity vs. distance (km) and days of transport	-0.572	0.003 *e*
biological similarity vs. days of transport and distance (km)	0.469	0.006 *e*
biological similarity vs. distance (km) and environmental factors	0.084	0.324 *e*
biological similarity vs. environmental factors and distance (km)	-0.402	0.019*
biological similarity vs. days of transport and environmental factors	0.150	0.219 *e*
biological similarity vs. environmental factors and days of transport	-0.537	0.008*

(*e*) spurious correlations, sensu Cramer (2003); (*) statistical significance p<0.05

A partial Mantel test indicated that the correlation of biological similarity with environmental factors, controlling the geographical distance and controlling for distance in days of transport, was negative and significant in both cases (Table 3). The other possible combinations were spurious according to Cramer (2003).

Discussion

Results obtained with the use of different techniques of classification and ordination enabled us to identify patterns of relationship between reef localities by their geographic range and to find results matching our working hypothesis, namely that closer localities are more similar to each other because they share a higher number of species rather than remote locations.

Cluster analysis showed an unexpected similarity between the Veracruz Reef System (VRS) with some more geographical distant reefs of the Mexican Caribbean. This is contrary to the expected idea that the VRS should be more similar to coral reef localities such as those in the Yucatan shelf or those in Northern Veracruz like Tuxpam or Lobos reefs, which are closer. One of the obvious reasons for this similarity is the number of shared species: of the 16 species of stony corals in the VRS, 14 of them are common or shared with Akumal and Mahahual in the Mexican Caribbean, while Cayo Arenas and Triangulos in the Yucatan platform, share only eight and five species with the VRS, respectively.

Higher similarity with the VRS, as well as the higher diversity of coral species at the regional level in the Mexican Caribbean, may indicate in the first case a historical accumulation of species richness in the context of higher stability associated with lower frequency of hurricanes in the Southwest Gulf of Mexico, whereas in the latter case, the recurrent impact of hurricanes in the Caribbean has caused a series of events of destruction and variable pulses of recruitment over time that have produced a diverse regional fauna

but a relatively low richness and low similarity scenario at the local level.

In the last 157 years (National Hurricane Center, 2008), the hurricane frequency as well as the accumulated impacts (frequency weighted by their intensity according to Saffir-Simpson scale) shows differences between regions in the study area. In the Gulf of Mexico, 17 hurricanes of a total of 28, had impacted the coral reefs on the Yucatan shelf, most of them with H1 category. Coral reefs in Northern Veracruz (Tuxpam and Lobos Island) had been impacted by twelve hurricanes, mainly H1 hurricanes, whereas the VRS has received only two impacts by hurricanes. On the Mexican Caribbean, coral reefs have been impacted by a total of 48 hurricanes, with the highest frequency in its northern portion with 28 hurricanes.

In the case of the coral reefs of the Mexican Caribbean and despite the observed patterns of association, there is a high heterogeneity in species composition, indicating that fewer coral species are shared among the sites in that region.

Within each geographic region, similarity between localities decreases with geographical distance, which is generally accepted by the current theory. Our results provide some evidence of the main patterns of connectivity amongst the reefs examined, concluding that ocean currents are the main driving factors responsible of the patterns shown; details of how other factors interact with interconnections of species and reefs are still unknown.

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Using Ichthyoplankton Distribution in Selecting Sites for an MPA Network in the Sulu Sea, Philippines

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Abstract. This investigation was conducted to provide data and information needed to establish an ecologically functional network of MPAs in the Sulu Sea Basin, by making use of data on fish larval (ontogenetic) distribution and composition, as well as dispersal modeling in identifying areas with potentially high recruitment (sink) and or areas with high potential as egg and larval sources. During the transition to the NE Monsoon (October), highest egg concentrations were observed in the immediate vicinity of the atolls and reefs along Cagayan Ridge and in embayments along the western border of Sulu Sea (east coast of Palawan), but larvae were most abundant along a north-south mid-basin transect from Cuyo Shelf. to Cagayan Ridge. Large-scale circulation shows that the Cuyo Group of Islands to be a major source of larvae to the western half of Sulu Sea, while the prevailing strong northeasterly current isolates Cagayan Ridge from Western Sulu Sea. The ontogenetic distributions of larval groups are consistent with the above scenario. The results are discussed in the context of connectivities within Sulu Sea and their implications to fisheries management.

Key Words: ichthyoplankton, connectivity, Sulu Sea

Introduction

Most shallow water fish species produce pelagic eggs and larvae (Bone et al. 1995). As such, these early life stages may be carried by ocean currents away from where they were spawned (dispersal) and or retained by local circulation (e.g., entrainment cells) within the vicinity of spawning grounds. The extent of dispersal/retention ultimately determines how important source-sink connectivity (Roberts and Polunin 1991; Ratikin and Kramer 1996) is to recruitment and in what spatial scales this is relevant. Making this a major consideration in establishing ecologically functional networks of marine protected areas enhances success of management efforts in the long run. In this context, examining the distribution of ichthyoplankton (fish eggs and larvae) and relating this to potential dispersal or transport of planktonic larvae as influenced by circulation patterns are of critical importance.

The Sulu Sea is a deep semi-enclosed basin whose connections with neighboring basins is limited to passes with very shallow sills (Menez et al. 2006), which also serve as important "marine corridors" for larval exchange. This study characterizes the distribution of fish eggs and larvae in Central Sulu Sea in relation to basin-scale circulation during the transition to the Northeast monsoon season.

Materials and Methods

The ichthyoplankton survey was conducted in October 2006 and covered the area of Central Sulu Sea from Panay and Negros Islands along the eastern border westwards to the eastern coast of Palawan (Fig. 1). Transects were strategically located to allow sampling across simulated current flow patterns (Villanoy et al. 2007) typical for this time of the year, the transition to the NE monsoon which is a major spawning season for many tropical marine organisms in the Philippines.

Stations were laid out approximately every 10-15nm along each transect. Samples were collected by means of 15 minute horizontal (sub-surface; within upper 1m) and double oblique tows (maximum targeted depth of 100m) using a 335 µm conical plankton net attached to a 60cm diameter ring. Mechanical flowmeters were mounted across the mouth of the net to measure the volume of water filtered. Horizontal tows were intended to sample fish eggs which are normally buoyant and inactive, and are thus generally found close to the surface. Double oblique tows, on the other hand, were used primarily to reduce the effect of time of day on the samples. Fish larvae and other plankton exhibit vertical movement in the water column in relation to light, affecting their vulnerability to plankton nets during the daytime. By employing a combination of tows, comparisons of estimates of egg and larval

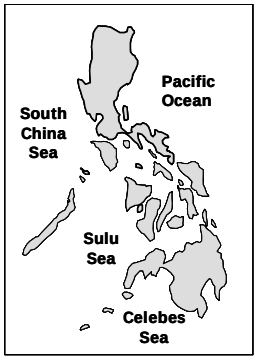
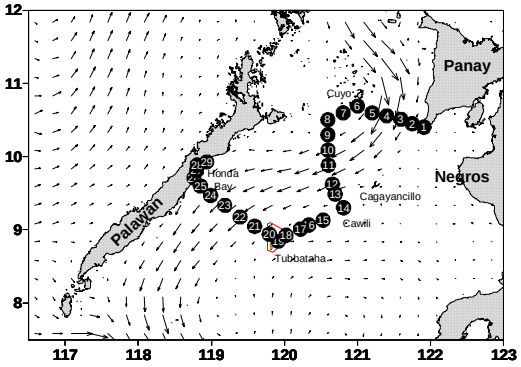


Figure 1. Map of the Philippines showing Sulu Sea (left) and the locations of stations surveyed in October 2006 (below). Note: arrows indicate the direction and relative magnitude of prevailing currents in the Sulu Sea in October (Villanoy et al., 2007)



concentrations at different times of the day can be made.

Samples were fixed in 10% buffered seawater-formalin solution in the field and brought back to the laboratory for sorting, identification and stage (developmental) determination.

Results & Discussion

Plankton Abundance and Distribution

The distributions of fish eggs and larvae are shown in Figures 2a and b. Across all 29 stations, mean larval density was 113.6 ind/100m^3, which is around 5 times the overall mean larval density recorded for the same general area in April 2006 (mean = 18.0 ind./100m3; sd 20.3; Campos et al., 2007). Moderate to high larval concentrations were recorded in the three transects traversing the main current flow, with highest densities (mean = 189.6 ind./100m^3; sd = 32.5) observed between Cuyo Shelf and Cawili Is.. Moderate larval densities were observed along the transect from the southern tip of Panay Is. west to Cuyo Shelf (mean = 123.1 ind./100m^3; sd = 52.0) and from mainland Palawan east to Tubbataha (mean = 103.0 ind./100m^3; sd = 55.8), respectively. The lowest densities (mean = 33.5 ind/100m^3; sd = 25.1) were recorded along the transect from Cawili Is. to Tubbataha (Fig. 2a).

Mean egg densities in October (mean = 2.1 eggs/m^3; sd = 4.9) were also higher by an order of magnitude than observed concentrations in April 2006 (mean = 0.29 eggs/m^3; sd = 0.45; Campos et al. 2007), although variability in both surveys was high. High variability is generally due to the patchy distribution of fish eggs. During the survey, high egg concentrations were recorded off the tip of Panay, in the Cuyo group of islands, Cawili Is., Tubbataha atoll, and Honda Bay in Palawan (Fig. 2b). Mean densities in these locations showed a mean of 5.7 eggs/m^3 (sd = 7.3), which is about 30 times the average densities in the rest of the other stations (mean = 0.2 eggs/m^3; sd = 0.12).

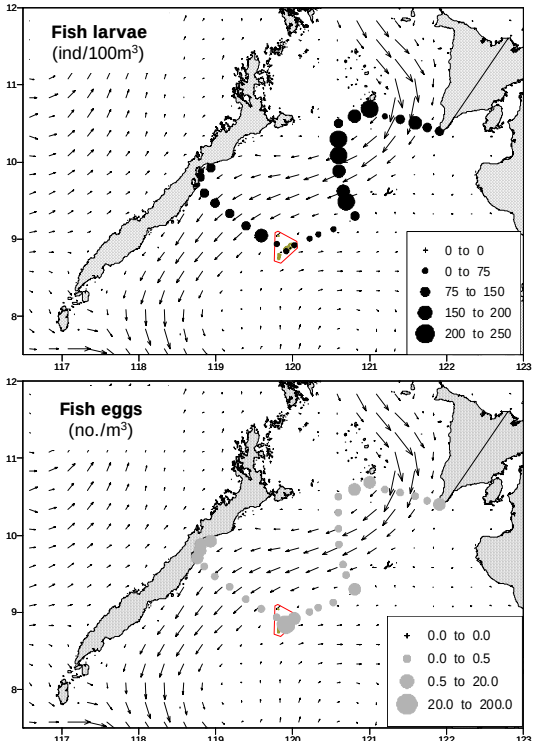


Figure 2. The density distribution of fish larvae (upper) and eggs (lower) in the Central Sulu Sea in October 2006.

Larval Assemblage Structure

A cluster analysis was performed on larval composition data from the 29 stations and the results are summarized in Fig. 3 and Table 1. Three major station clusters were formed by the analysis: Palawan Coast, Cagayan Ridge (Tubbataha to Cawili), and stations within the main flow. The latter group was further subdivided into a sub-group along the eastern margin of the main flow, and another within the main axis. The relative composition of larval assemblages in these clusters is shown in Table 1. The cluster of shallow stations along the coast of Palawan is dominated by larvae of soft-bottom demersal fish,

such as gobiids, nemipterids and cynoglossids. On

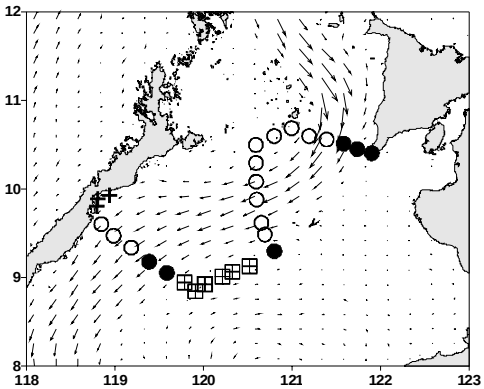


Figure 3. Location of station clusters in the Central Sulu Sea in October 2006: Palawan coast (+), Cagayan Ridge (⌗), Main flow (○), and main flow margin (●).

the other hand, the Cagayan Ridge cluster of deep

Table 1. Relative abundance (%) of the various larval groups in the four station clusters.

Larval groups	Palawan Coast	Cagayan Ridge	Main Flow Margin	Main Flow
Demersal	67.0	12.1	11.1	21.3
Mesopelagic	3.8	44.8	57.0	22.7
Reef-associated	23.5	23.2	17.0	41.0
Epipelagic	1.3	5.3	8.7	8.7
Unidentified	4.3	14.5	6.2	6.3
	100	100	100	100

open water stations was dominated by larvae of mesopelagic fish, such as myctophids, gonostomatids and paralepidids, but showed substantial contributions (23.2%) from reef-associated groups like scorpaenids and balistids. Reef-associated larvae in this cluster likely originate from the atolls or islands along the Ridge. The stations of the main current flow showed assemblages comprised primarily of reef-associated and mesopelagic fish larvae. These stations are in deep open water and this explains the abundance of mesoplegaic fish larvae which are likely spawned in open water. However, because reef fish generally spawn in reef areas, their abundance in this cluster indicates their origin from sources upstream of the main current flow, like Cuyo Shelf, where extensive shallow reef areas are located. Among stations in this cluster, the ones along the eastern margin of the main flow show much larger contributions from mesopelagic fish larvae (57%) than stations within the main axis of the flow (22.7%).

Ontogenetic Distribution

The ontogenetic distribution of reef-associated larvae show a progression from higher upstream concentrations in younger (preflexion) stages to higher downstream concentrations in older (postflexion) stages (Figs. 4a-c). A similar pattern was shown by larvae of demersal fish. These results are consistent with spawning in Cuyo Shelf and subsequent transport towards the southwest in the direction of the main flow as larvae age and develop. In contrast, mesopelagic larvae do not show any progression of concentrations with age (Figs. 5a-b). This may be attributed to more open and deep water

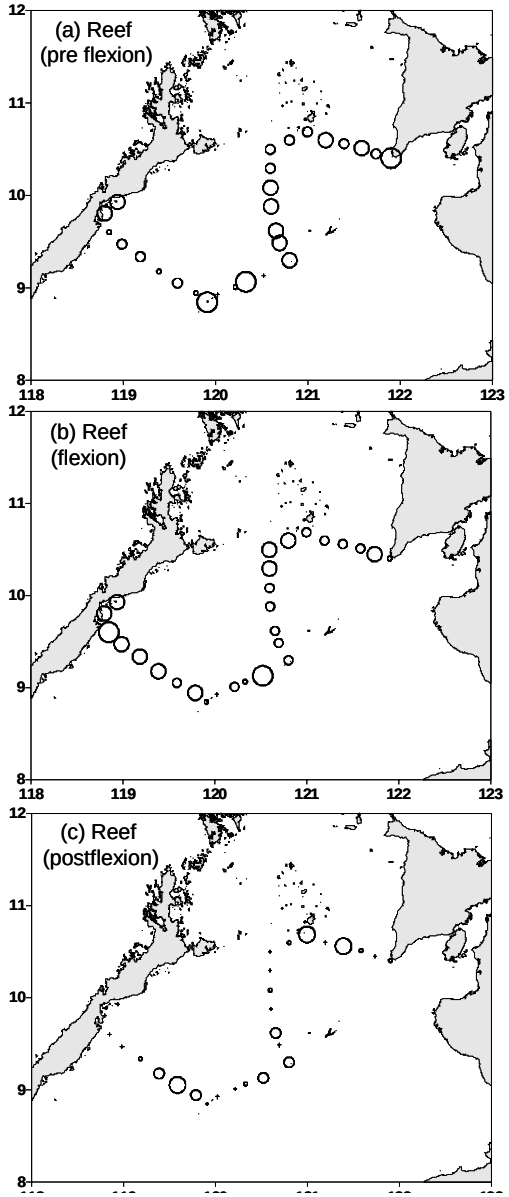


Figure 4. Ontogenetic distribution of larvae of reef-associated fish in the Central Sulu Sea in October 2006.

spawning grounds of these fish, which covers most of the Sulu Sea. No examination of epipelagic larvae was done due to their low number in the samples.

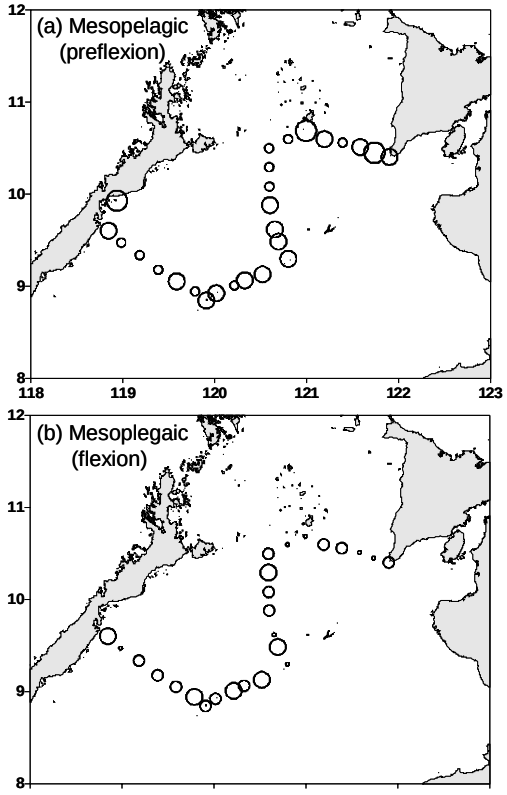


Figure 5. Ontogenetic distribution of larvae of mesopelagic fish in the Central Sulu Sea in October 2006.

Larval distribution patterns and hydrography

Large scale circulation in the Sulu Sea in October is dominated by a strong current flowing from the northeast and then southwards along the coast of Palawan (Fig. 1). This current covers a wide path, extending from Cuyo Shelf all the way to Panay, and from the east coast of Palawan to just west of Cawili Island. Because of the relatively strong current in the main axis, it is unlikely that plankton are able to cross it. This effectively isolates the atolls and islands of Cagayan Ridge from western Sulu Sea, at least during this time of the year. However, entrainment further upstream may result in downstream dispersion within the flow.

A review of egg and larval distributions in relation to large-scale water movement allows scenario-building. The patchy larval concentrations along the transect from Cuyo to Panay (Fig. 2a) would be consistent with spawning at the endpoints of this transect, as also shown by egg concentrations at these locations (Fig. 2b), and not further north. If major spawning had taken place further north of Cuyo Shelf, entrainment in the strong southward current would have caused larval concentrations along this entire transect to be more homogeneous. On the other hand, major spawning in the Cuyo group of islands would still be delimited by the south-flowing current in the area near Panay, but entrainment would result in high and relatively homogeneous larval concentrations in transects further downstream (i.e., Cuyo to Cawili), as was observed during the survey (Fig. 2a). The preponderance (> 70%) of shallow water fish larvae (e.g., demersal, epipelagic and reef-associated) within the main flow (Table 1) is consistent with Cuyo Shelf being the major source. The reduced larval concentrations between Tubbataha and mainland Palawan may be due to larval settlement (recruitment) along the stretch of Palawan's coastline north of Honda Bay.

The difference in assemblage composition between the cluster of stations along the eastern margin of the main flow and those within the main axis of the flow is consistent with Cagayan Ridge being isolated from Western Sulu Sea during this time of the year. Spawning grounds along the Ridge, as indicated by egg concentrations (Fig 2b), will likely be self-feeding, at least during this season, with apparent transport being facilitated by a weak gyre formed by currents immediately west of the Ridge flowing towards the southwest and those immediately east flowing towards the opposite direction. Again, the similarity in taxonomic make-up of assemblages in the main flow margin and the Ridge (i.e. > 44% mesopelagics; moderate amounts of reef-associated larvae; Table 1) is consistent with this scenario.

Implications for fisheries management

For the Central Sulu Sea, the extensive Cuyo Shelf area appears to be a major source of larvae for habitats along the East Coast of Palawan, and perhaps even further south, at least during the transition to the NE monsoon. Improving protective management efforts in Cuyo Shelf would benefit an extensive area downstream. Also during this season, spawning and recruitment within Cagayan Ridge seem to be limited locally. Hence, management efforts here would have little impact on fishing to the west, although the situation will likely be different in other seasons. What happens during the rest of the year is still largely unknown but on-going analyses of survey data from the Summer (April) 2007 will provide more information on this.

While it is believed that dispersal from MPAs with increased spawning biomass is the mechanism that enhances sustainability of fishing downstream,

unequivocal empirical evidence for this is still largely unavailable (Russ 2002). The results of this study do not provide such data, but they indicate which portions of the Central Sulu Sea are functionally connected during the transition season to the NE monsoon, and where improved management can be focused.

There are currently over a thousand marine protected areas in the Philippines, with more than 1/3 covering less than 10 ha. Recent figures show that only about 10% of these are properly managed (Campos and Alino 2008), due primarily to scarce support resources that are also spread out thinly. Under such circumstances, information provided from this study provides a strategic framework for optimizing management efforts with enhanced prospects for long term success.

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Genetic structure of the scleractinian coral, *Pocillopora damicornis,* from the Mexican Pacific

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Abstract. Genetic structure was studied in the coral *Pocillopora damicornis* in three areas of coral development of the Mexican Pacific (MP). Specimens were collected from six localities: El Portugues and Punta Gaviotas located inside the Gulf of California (GC), Punta Arenas and Isla Redonda from the entrance of GC, and Las Dos Hermanas and La Entrega in the south of the MP. Exclusive genotypes were observed in the northern and southern populations. Genetic variation was similar along the MP, although slight higher variation was observed in the southern populations. Most of the populations presented significant deficits heterozygotes. These deficiencies could be due to the predominance of the asexual reproduction by fragmentation, localized sexual reproduction, inbreeding and/or Wahlund effect among localities, and different mortality events by natural disturbances. Cluster analysis based on genetic distance showed three groups by geographical proximity: two populations inside the GC, those ones located in the entrance of the GC, and two populations located at the south of the MP. Mean significant F_{ST} value (F_{ST} = 0.153) indicated a genetic structure. Differences in sexual and asexual reproduction among the localities coupled with local recruitment and currents patterns are possibly generating the genetic structure observed in the populations of *P. damicornis* in the MP.

Key words: Population structure, *Pocillopora damicornis*, Mexican Pacific, population genetics.

Introduction

The coral *Pocillopora damicornis* is one of the dominant coral species in reef systems of the eastern Pacific. This coral is distributed from the Gulf of California in Mexico to Ecuador, including all the nearby oceanic islands (Glynn and Ault 2000; Pérez-Vivar et al. 2006). This species is one of the most studied and widely distributed reef corals in the world. Examination by allozyme electrophoresis of this species has allowed determinate the genetic structure in coral populations throughout the Indo Pacific (Stoddart 1984; Ayre *et al*. 1997; Yu *et al*. 1999; Ayre and Hughes 2004; Smith-Keune and van Oppen 2006).

In the Mexican Pacific (MP), reproductive studies indicated that this species can reproduce both sexually (spawn gametes) and asexually (fragmentation) and presents a geographic variation in its reproductive mode along the Mexican Pacific (Chávez-Romo and Reyes-Bonilla 2007; Carpizo-Ituarte *et al*. 2009). In addition, there is not information about the genetic variation of *P. damicornis* in the MP. Genetic studies realized in other coral species as *Porites panamensis* (Paz-García *et al*. 2009b) and *Pavona gigantea* (Saavedra-Sotelo 2007) have showed a genetic structure in their populations.

Our aim was to determine the genetic structure of the coral *P. damicornis* along of the Mexican Pacific. This study is relevant due the populations are in active recovery after bleaching and mortality caused by the 1997-98 El Niño event (Carriquiry *et al*. 2001; Reyes-Bonilla 2001).

Material and Methods

Sample Collection. We collected 22 to 48 fragments (2 m of depth) of *P. damicornis* from six localities along of the MP (Fig. 1): El Portugues (POR) and Punta Gaviotas (PGA) inside of the GC (GC); Punta Arena de la Ventana (PAV) and La Isla Redonda (IRD) in the entrance of the GC; and two localities from the south of MP, Las Dos Hermanas (LDH) and La Entrega (LET). The coral fragments were frozen in

liquid nitrogen and transported to the laboratory, where they were stored at -80°C.

Figure 1. Collection areas. Points indicate collected sites along the Mexican Pacific. **POR** El Portugues, **PGA** Punta Gaviotas, **PAV** Punta Arenas, **IRD** Isla Redonda, **LDH** Las Dos Hermanas and **LET** La Entrega.

Electrophoresis. We conducted a coral tissue extraction in Stoddard's buffer modification (Stoddart 1983, Weil 1992) using a sonic desmembrator. Homogenates were centrifuged at 2600 g for 10 min at 4 °C, and the supernatants were stored at -80 °C. We determinate the concentration of total proteins from each sample by Bradford's method (Bradford 1976) and 25 to 50 µg of sample was used for the analysis of each enzyme system (Paz-García et al. 2009a). Allozyme analysis was carried out using vertical electrophoresis with 8%T polyacrilamide gels (Manchenko 1994). Four enzyme systems were used: Leucyl glycyne glycyne peptidase (LGG 1, E.C. 3.4.11.1) enzyme malic (ME 1&2, E.C. 1.1.1.40), glutamate dehydrogenase (GDH 1, E.C. 1.4.1.3) and esterase (EST 1&2, EC 3.1.1.1). Tris-Glycyne Buffer was used in the electrophoresis. Alleles were assigned a value based on the ratio of their electrophoretic mobility relative to that of the most common allele.

Statistical analyses. Genetic variability was calculated for each population and the Hardy-Weinberg equilibrium (HWE) was tested by χ^2 analyses, using the Biosys-1 and GENPOP programs (Swofford and Selander 1981; Raymond and Rousset 1995). The magnitude and direction of departures from HWE at each locus were also assessed to each population. These departures were expressed as Wright's fixation index (*f*), where positive and negative values represented deficits and excesses of heterozygotes, respectively (Wright 1978). Unbiased genetic distance was used for cluster analysis, calculated in TFPGA software (Nei 1978; Miller 1997). Wright's *F* statistics were calculated to determine the degree of differentiation among populations (Weir and Cockerham 1984). We calculated pairwise F_{ST} estimates between each pair of populations. F_{ST} were tested for difference from zero permuting (10 000 replicates) alleles between samples with exact G-test (Goudet *et al*. 1996), as implemented in FSTAT v. 2.8 (Goudet 1995). We applied a sequential Bonferroni correction to reduce the chance of type I errors (Rice 1989).

Results

Six loci were scored from four enzyme systems, one monomorphic and five polymorphic. Exclusive genotypes were observed in the northern (POR: LGG-1^{AB}; PGA: LGG-1^{AB} and LGG-1^{BC}) and southern (LDH: LGG-$1^{AC,}$ LGG-1^{BC} and LGG-1^{CD}; LET: LGG-1^{AC} and LGG-1^{CD}) populations (Anexus I). Allelic diversity was 2.3 in the populations from inside and entrance Gulf of California and 2.5 in south of MP (Fig. 1a). Genetic variation was slight higher in southern populations (LDH and LET). All populations presented significant deviations of Hardy-Weinberg equilibrium in deficits of heterozygotes (Fig. 1b and Table 1).

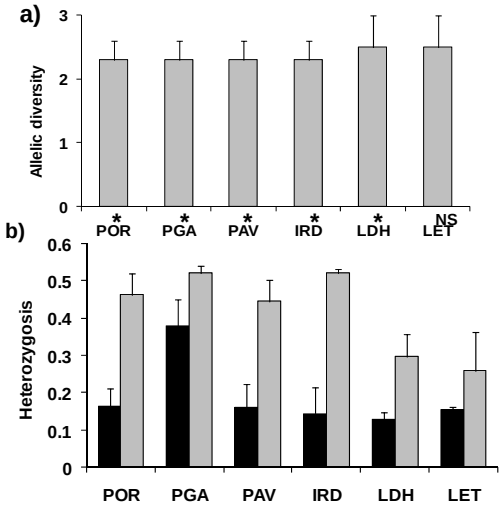

Figure 2. **a**) Allelic diversity (calculated with 5 loci polymorphic). **b**) Heterozygosis observed and expected, black and gray respectively, in six populations of *Pocillopora damicornis*. Error bars indicate (SE) variation among populations. Significant level after sequential Bonferroni correction (*$p < 0.001$). NS: Not significant.

Table 1. Wright's fixation index (*f*) indicating heterozygote excess (negative number) or deficit (positive number) for each locus in populations of *P. damicornis* from the Mexican Pacific. Significant deviations from Hardy–Weinberg equilibrium after sequential Bonferroni correction (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Population	POR	PGA	PAV	IRD	LDH	LET
Locus						
ME-1	0.672***	-0.080	0.523**	0.894***	0.760**	-0.077
EST-1	0.300	0.107	0.414	0.506	0.468	-0.100
EST-2	0.467*	0.275	0.513*	0.417	-0.077	-0.083
GDH-1	1.000***	1.000***	1.000***	1.000***	1.000***	1.000***
LGG-1	0.959***	0.675***	1.000***	1.000***	0.696***	0.759*

The UPGMA cluster analysis, based on Nei's (1978) unbiased genetic distance, showed three groups by proximity geographical: I) the populations from inside of the GC, II) two populations from the entrance of the gulf and III) the populations of the south of MP as other cluster (Fig. 2). The individual inbreeding coefficient (F_{IS}) and the total inbreeding coefficient (F_{IT}) were significantly different from zero in each locus and mean value (Table 2). F_{ST} values were statistically significant, except for locus EST-2, and ranged from 0.062 to 0.213. Mean significant F_{ST} value (F_{ST}=0.153) indicate a population structure of *P. damicornis* in the MP (Table 2).

F_{ST} values calculated between pairs of populations, ranged from 0.027 to 0.420 (Table 3). These comparisons showed a great genetic subdivision of the populations from LDH and LET with the other analyzed populations (Table 3).

Table 2. Wrights *F*-statistics calculated to each locus for *P. damicornis* from the Mexican Pacific. Significant level after sequential Bonferroni correction (*$p < 0.05$, **$p < 0.01$).

Locus	F_{IS}	F_{IT}	F_{ST}
ME-1	0.405**	0.447**	0.062*
EST-1	0.294**	0.429**	0.184*
EST-2	0.422**	0.443**	0.040^{NS}
GDH-1	1.000**	1.000**	0.213*
LGG-1	0.837**	0.866**	0.187*
Total	0.625**	0.682**	0.153*
Average Jacknife	0.632	0.691	0.155
Standard error	0.140	0.127	0.030
Bootstrap over loci 95% Confidence Interval	0.374-0.862	0.456-0.887	0.096-0.200

Table 3. Pairwise F_{ST} (below diagonal) and *p* values (above diagonal) in *P. damicornis* from the Mexican Pacific. Significant level after sequential Bonferroni correction (*$p < 0.01$). ^{NS}: Not significant.

Population	POR	PGA	PAV	IRD	LDH	LET
POR	———	0.039^{NS}	0.002^{NS}	0.025^{NS}	0.000*	0.000*
PGA	0.027	———	0.007^{NS}	0.004^{NS}	0.000*	0.000*
PAV	0.048	0.034	———	0.148^{NS}	0.000*	0.000*
IRD	0.057	0.065	0.034	———	0.000*	0.000*
LDH	0.361	0.244	0.362	0.420	———	0.014^{NS}
LET	0.232	0.135	0.188	0.265	0.148	———

Discussion

Allelic diversity in most populations of *Pocillopora damicornis* from the Mexican Pacific showed slight lower values (2.2-2.5; Fig. 2a), compared with those reported in six populations from Japan which ranged of 3.1 to 3.8 and six habitats from One Tree Island Reef, Australia with values from 3.5 alleles per locus (Adjeroud and Tsuchiya 1999; Sherman et al. 2005). In addition, a declination of allelic diversity with increasing latitude (from 3.6 to 1.3) was observed in *P. damicornis* along the east of mainland Australia. This variation suggests a high genetic differentiation among the populations (Miller and Ayre 2008). Therefore, the observed values of allelic diversity suggest a lower genetic differentiation among the populations from the PM.

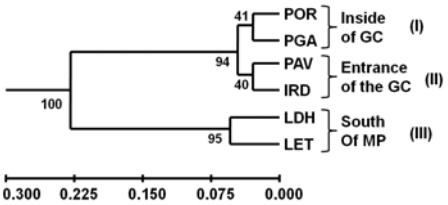

Figure 3. Cluster analysis based on Nei's (1978) unbiased genetic distances showing relationship among populations of *Pocillopora damicornis* from the Mexican Pacific. Bootstrapped values over 40% (based on 1000 randomizations) are shown next to corresponding nodes.

The slight higher genetic variation and exclusive genotypes found in the southern populations of *P. damicornis*, could be due to a larval dispersion coming from the populations from Central America (Panama, Costa Rica and Galapagos Islands). Possibly originated by a high genetic diversity in those populations that perhaps have a past genetic contribution of the populations from the Indo Pacific. In addition, recent studies realized in Panama, Clipperton Island and Hawaii, indicate that trans-Pacific gene flow in *P. damicornis* between the Central and Eastern Pacific is restricted; and the Eastern Pacific corals exist in relative isolation from their Central Pacific counterparts and interact with each other differently (Combosch *et al*. 2008).

The observed heterozygosis values ranged from 0.127 to 0.378 (Fig. 2b) and they are similar to six populations from Japan that present an asexual reproduction as main mode in the maintenance of their populations (ranged from 0.244 to 0.366; Adjeroud and Tsuchiya 1999). We detected significant deviations from HWE in each population (Table 1). Significant heterozygous deficit has been commonly observed in coral species (Adjeroud and Tsuchiya 1999; Nishikawa and Sakai 2005; Miller and Ayre 2008; Paz-García et al. 2009). These deviations has been associated to the predominance of the asexual reproduction by fragmentation, inbreeding and/or Wanhlund effect among localities, as consequence of different events of recruitment of different cohorts and for different coralline communities, and different mortality events by natural disturbances (Ayre et al. 1997; Adjeroud and

Tsuchiya 1999; Sherman et al. 2005; Ayre and Hughes 2004; Constantini et al. 2007).

Cluster analysis based on Nei's (1978) unbiased genetic distance suggests that the populations of *P. damicornis* that are from inside the GC (POR and PGA), and the entrance (PAV and IRD) of the GC, possess slight genetic differences (Fig. 3). While the LDH and LET present high genetic differences with northern populations. This pattern suggests a subdivision among the populations of *P. damicornis* and coincides with the observed in *Porites panamensis* along the MP (Paz-García *et al*. 2009b). These results suggests that populations from inside and the entrance of GC may be a group genetically more homogeneous, while that southern populations showed a genetic differentiation that could be due to the high frequency of natural phenomenon that they are generally presented along the MP (e.g. hurricanes, torments, ENSO events, upwelling areas; Medina-Rosas *et al*. 2005; Paz-García *et al*. 2009b). Furthermore the values of the bootstrapped in the cluster analysis corroborate this differentiation in the most southern populations (Fig. 3) and it coincides with that found in other coral species studied in the MP as *P. panamensis* and *P. gigantea* (Saavedra-Sotelo 2007; Paz-García *et al*. 2009b).

Significant differences found in the individual inbreeding coefficient (F_{IS}) and the total (F_{IT}) of the populations (Table 2), indicate a heterozygote deficiency and a possible local recruitment in the populations of *P. damicornis* in the PM. F_{ST} values in the most loci and mean significant F_{ST} value (F_{ST} = 0.153) indicated a high genetic structure in the populations of *P. damicornis* in comparison with other coral species studied in the MP as *P. panamensis* (F_{ST}=0.104; Paz-García et al. 2009b) and *P. gigantea* (Φ_{ST} 0.10-0.20; Saavedra-Sotelo 2007). Previous studies in the GC and MP have found population subdivision in different marine invertebrates and fishes groups (De la Rosa-Vélez et al. 2000; Riginos y Nachman 2001; Valles-Jimenez et al. 2005; Paz-García *et al*. 2009b). Different factors can generate a population genetic subdivision in the MP, as biogeography, distance geographic, habitat discontinuities, temperature gradients and upwelling areas (De la Rosa-Vélez et al. 2000; Riginos and Nachman 2001; Halfar et al. 2005; Valles-Jimenez et al. 2005).

In addition, the genetic differentiation observed in populations of *P. damicornis* could be due to differences in sexual (spawning gametes) and asexual (fragmentation) reproduction among the localities of the MP (Chávez-Romo and Reyes-Bonilla 2007; Carpizo-Ituarte *et al*. 2009). Studies realized in Japan and Australia have been reported differences in the reproductive strategies of *P. damicornis* and the main reference that the asexual reproduction contributes substantially in the genetic structuring and in the maintenance of established population of the species (Stoddart 1984a, b; Adjeroud and Tsuchiya 1999). Fragmentation has been observed as common reproductive strategy in the populations of the *P. damicornis* in the MP. Patches of this species easily colonize soft substrates (sand or gravel) after their branches become detached following cyclones; these branches attach themselves to the bottom and continue developing (Reyes-Bonilla 2003). However, recent studies show that the population of PGA present evidences of sexual reproduction (Chávez-Romo and Reyes-Bonilla 2007), which would explain because the populations of inside the GC have a close genetic relationship with the populations the entrance of the GC. Although previous studies mention that the population from IRD presents an asexual reproduction for fragmentation way (Carpizo-Ituarte *et al*. 2009).

Nevertheless, although the reproduction influences in the genetic differentiation, it is not considered to be the only factor. The local recruitment coupled with currents patterns may limit the larval dispersion and generate the genetic structure observed in the populations of *P. damicornis* in the MP. The possibility has been mentioned that the Coastal Current Costa Rica (CCCR), in direction south to north, transports coralline propagules from Central America until Oaxaca, for the likeness of coralline species among these areas (Glynn and Wellington 1983; Reyes-Bonilla and López-Pérez 1998). Also, dispersion may occur from Oaxaca to Bahia de Banderas and from there to the Gulf of California. However the exclusive genotypes found in the OAX populations (LDH: LGG-$1^{AC,}$ LGG-1^{BC} and LGG-1^{CD}; LET: LGG-1^{AC} and LGG-1^{CD}) indicate that not all the coral species can be dispersed in the same way because different oceanic fronts exist to the south of Bahia de Banderas and the Gulf of California and that which coincides with that observed for *P. panamensis* along the MP (Reyes-Bonilla 2003; Paz-García *et al*. 2009b). Also, recent studies of the reproductive biology of *P. damicornis* in the population LET showed evidences that these reproduce in an asexual way (fragmentation; Carpizo-Ituarte *et al*. 2009), that which would support the fact that the genotypes found in LDH and LET were exclusive of these populations due a dispersion capacity doesn't exist toward the north. The dispersion could be limited between coral population with sexual reproduction by the existence of long sand barriers and mangroves communities localized along the MP (Glynn y Ault 2000; Reyes-Bonilla 2003). However, it is necessary to carry out other studies that analyze the ecology, reproduction and genetics of the populations of *P. damicornis* to

understand the relationship that exists among these ecosystems along Mexican Pacific.

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South east African, high-latitude coral communities, a canary for western Indian Ocean coral reefs?

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Abstract. Whether south west Indian Ocean reef coral communities are resilient to anthropogenic and natural stressors will depend on their inherent ability to adapt to change. In this study, reef coral population diversity and relatedness were investigated at varied scales using molecular methods. Genetic diversity may be used as a proxy to gauge both the population dynamics and resilience of a community and was thus measured in two corals with different reproductive modes, larval dispersal capabilities and life history strategies. We used an intron region in the nuclear DNA of *Acropora austera* and the ITS region of the ribosomal DNA of *Platygyra daedalea* to compare genetic variability between populations. In *A. austera* there appears to be regular genetic exchange between populations in the region. The ITS region has, however, been demonstrated to yield inconclusive results. Local oceanography dictates that northern reef systems may be considered the source populations of those in the south. However, local reef-coral genetic diversity and connectivity may be better judged with a combination of finer resolution molecular markers.

Key words: Indian Ocean, reef-coral, genetic diversity, connectivity.

Introduction

Many scleractinian corals produce propagules which may move between habitats suitable for recruitment in a mobile pelagic stage which has potential for long-distance dispersal. Consequently, initial hypotheses considering larval viability proposed large genetically-unstructured populations for these marine invertebrates (Avise 1998). However, measuring gene flow as a proxy for movement using allozyme studies has confirmed that although some populations of hard coral showed high levels of gene flow, they also had structured populations (Ayre and Hughes 2004, Dai *et al.* 2000, Goffredo *et al.* 2004, Hellberg 1994, Nishikawa and Sakai 2005).

Various studies of genetic variability have revealed the relatedness of life strategy to connectedness of populations (Hellberg 1994; Bastidas *et al* 2002; Goffredo *et al*. 2004; Miller and Ayre 2004, LeGoff-Vitry *et al.* 2004), population structure (Mackenzie *et al.* 2004), speciation (Fukami *et al.* 2004, Marquez *et al.* 2002) and reticulation within species (Diekmann *et al.* 2001). Genetic information may reveal unexpected patterns of population structure and gene flow in hard reef corals and thus is a powerful tool in their management.

A recent review showed that little genetic work has been conducted on marine organisms in the south west Indian Ocean (SWIO) (Ridgway and Sampayo 2005). A number of studies have subsequently been published which shed light on patterns of connectivity and structure in scleractinia and their symbionts in the SWIO (Ridgway *et al.* 2008; Souter and Grahn 2007; Mangubhai and Souter 2007; Macdonald *et al.* 2008). Oceanic waters of Maputaland in the northern KwaZulu-Natal province of South Africa harbour the southern-most communities of coral in the SWIO (see Fig. 1). These communities comprise approximately 90 species of coral (hard and soft) which form the basis for these diverse communities (Schleyer 1999). Further north, the Bazeruto Archipelago also supports a diverse assemblage of corals on accretive reef systems. A gradient of species diversity decreasing from north to south (Obura 2000), along with predominantly southerly offshore currents in the Mozambican channel (5cm.s^{-1}, Lutjeharms 2006) implies that the more diverse northern reefs may be seeding the southern reef coral systems. This, however, has not been tested with appropriate genetic marker systems. As less than 2% of the coast is under any sort of management (Wells and Ngusaru 2004), regional authorities are developing management plans for local exploited resources. We sampled the east African coastline from a number of sites between northern Maputaland, South Africa and southern Tanzania. We collected *Acropora austera*, a widespread coral with a broadcast-spawning reproductive strategy common to the scleractinia (Carroll *et al*. 2006). We also sampled a Favid,

Platygyra daedalea, which, although also a broadcast spawner, displays a life strategy with slower growth and a pattern of intra-reef dispersal different from that observed in *A. austera* (Miller and Babcock 1997).

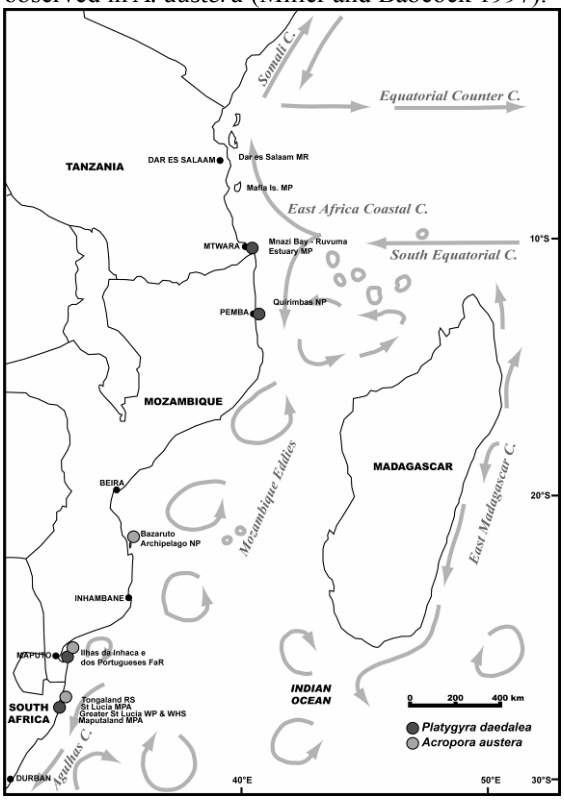

Figure 1. Currents, eddies and sampling locations of *A. austera* and *P. daedalea* in the SWIO study area.

*W*e used nuclear intron sequences similar to those developed by Concepcion *et al.* (2007) for *A. austera* specimens and ITS sequences for *P. daedalea*. ITS rDNA, however, is subject to concerted evolution, the rate of which may either obscure (Vollmer and Palumbi 2004) or resolve phylogenies (Lam and Morton 2003) and introgressive recombination (Vollmer and Palumbi 2004, Diekmann *et al.* 2001) which may further obscure inter-species variation and inter-population variation. This has, however, only been shown in certain families of the scleractinia (*Acroporidae*) (Vollmer and Palumbi 2004) whereas analysis of this region has revealed significant population differentiation in the *Faviidae* (Rodriguez-Lannetty and Hoegh-Guldberg 2002, Lam and Morton 2003), particularly within the genus *Platygyra*. We sequenced specimens from each collection locality using nuclear markers to obtain an initial estimate of nuclear genetic variability.

Materials and Methods

Corals were sampled from sites along the east African coast, using SCUBA and snorkel diving. Care was taken to avoid the collection of clone-mates by sampling colonies separated by at least 5m. Samples were immediately stored in either a dimethyl sulfoxide (DMSO) salt buffer or 70 % ethanol. All DNA was isolated using a Fermentas Life Sciences^{TM} genomic DNA purification kit as per their extraction protocol.

Acropora austera DNA was amplified using single-copy nuclear intron primers developed at the Centre for Marine Studies (CMS) at the University of Queensland (Ridgway, unpublished data). These amplify a hypervariable intron region of the carboxy-anhydrase gene. PCR reactions contained: 1µl sample template, 21.68µl dH_2O, 3µl 10X Platinum Taq PCR buffer mix, 0.9µl 50mM $MgCl_2$, 0.6µl 40mM dNTP mix, 0.84µl of each primer (10µM) and 0.12µl Platinum Taq 5u/µl (Invitrogen^{TM}) . The following thermal cycle was used for the PCR: [94°C for 2 minutes], 40 X [(94°C for 60 seconds), (51°C for 60s), (72°C for 2 m)], [72°C for 10m], [10°C∞]. Samples were sequenced on an ABI 3730 capillary sequencer at Inqaba Biotechnology^{TM}.

Platygyra daedalea DNA was amplified using the A18S and ITS4 primers developed by Takabayashi *et al.* (1998). PCRs contained: 1µl specimen DNA, 21.68µl dH_2O, 3µl 10X supertherm Taq PCR buffer mix, 1.8µl 25mM $MgCl_2$, 0.6µl 40mM dNTP mix, 0.84µl of each primer at 10µM concentration and 0.24µl supertherm Taq. The following thermal cycle was used for PCR amplification: [95°C for 10m], 40 X [(94°C for 45s), (51°C for 45s), (65°C for 1m)], [72°C for 10m], [10°C∞]. Sequencing showed that some specimens were polymorphic and that cloning the samples would be necessary in order to obtain good quality sequence data. Samples were cloned at Inqaba Biotechnology, Pretoria SA, and re-sequenced.

*A*ll sequences were aligned using Bioedit, a sequence alignment package (Hall 1999). Sequence variation was compared using Arlequin 3.11 (Excoffier *et al.* 2005) and DNAsp (Rozas *et al.* 2003). A minimum spanning network was constructed in Arlequin 3.11 (Excoffier *et al.* 2005) for *A. austera* haplotypes and drawn using MrEnt 2 (Zuccon and Zuccon 2008).

Results

*S*equencing - Samples were collected from throughout the study area (Fig. 1, Tab. 1). *A. austera* carbonic anhydrase intron DNA sequences were 155 base pairs (bp) long, had a GC composition of 43.5% and showed a total of 12 variable sites among all 51 sequences. These 51 sequences comprised 9 haplotypes, with haplotype diversity (hd) of 0.697 (std dev = 0.064), which were shared amongst the populations studied (Tab. 2). The complete ITS region (ITS1-5.8S-ITS2) was sequenced for 22 *P.*

daedalea samples. The total sequence length was 374 bp whilst the G+C content was 58.3 %. These 22 sequences comprised 15 haplotypes with hd = 0.939 (std. dev. = 0.029) and $\pi = 4.9$

Table 1. Number of specimens from each site along the east African coast analysed in this study.

Sampling location	*Acropora austera*	*Platygyra daedalea*
Two-mile reef	18	3
Nine-mile reef		2
Inhaca Island – Bareirra Vermelho	6	6
Inhaca Island – Baixo Danae	13	
Bazeruto Island Archipelago	14	
Pemba Bay		6
Mtwara		5

Table 2. *A. austera* haplotypes distributed amongst sampled sites along the east African coastline (Tmr1 = Two-mile reef site 1, Two-mile reef site 2, Bd = Baixo Danae, BV = Barreira Vermelho, Bztmr = Bazeruto Two mile reef and Bzlh = Bazeruto Lighthouse reef).

Haplotype:	Tmr1	Tmr2	Bd	Bv	Bztmr	Bzlh
Hap_1	1		1	1	1	1
Hap_2	7	4	8		6	2
Hap_3	1				1	
Hap_4	1	2	2		1	
Hap_5		1	1		1	
Hap_6		1	1	1		
Hap_7				1		
Hap_8				2	1	
Hap_9				1		
Π	0.711	1.821	1.410	5.8	2.764	0.667

For *A. austera,* analysis of molecular variance (AMOVA) carried out in Arlequin 3.11 (Excoffier *et al* 2007) yielded $F_{ST} = 0.18$ ($p < 0.01$).
Barreira Vermelho (BV) was, however, found to show significant pairwise Fst values; when it was removed from the analyses the AMOVA showed no significant structure at any level. DNAsp was used to calculate the average number of migrants per generation ΔNm (Nei 1982) = 0.71 and the inter-populational gene diversity γst = 0.26. A minimum spanning network (MSN) was constructed to illustrate relationships between haplotypes and their distribution amongst populations (Fig. 2).

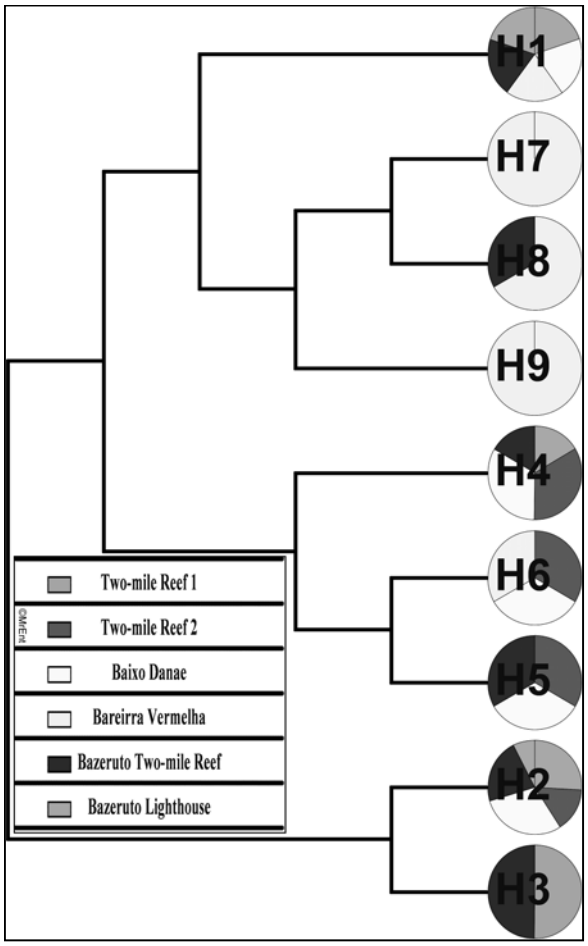

Figure 2 Minimum spanning network shows relationships between *A. austera* nuclear intron haplotypes and collection localities in the south-west Indian Ocean.

Using mismatch distributions (Fig. 3) we found that the Inhaca Island population of BV may have established equilibrium in terms of growth, in contrast to other populations (Rogers and Harpending 1992).

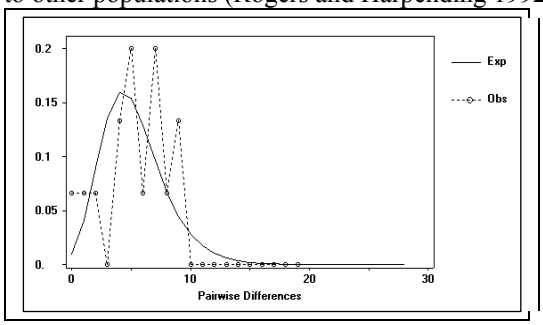

Figure 3. Pairwise mismatch distribution for population BV, generated in DNAsp (Rozas *et al* 2003).

Upon removing BV from further analyses, we found Nm = 3.87 and γst = 0.06, indicating that the remainder of the populations were less differentiated

from one another than they were from BV (Hudson *et al.* 1992). Genetic fixation indices calculated in AMOVA for *P. daedalea* were not significant. The proportional inter-population gene diversity was found to be γst = 0.29 with Nm = 0.63. We found evidence for a minimum of two recombination events within the ITS region of *P. daedalea*.

Discussion

Nuclear genetic markers from *A. austera* and *P. daedalea* showed markedly different levels of variation amongst the populations sampled, which may be attributed to the different evolutionary constraints under which they evolve. Analysis of sequence variation in the intron that was used to compare populations of *A. austera* showed that populations regularly exchange genes, with the exception of the population of Barreira Vermelho (BV), which may be isolated from the other local populations. Genetic diversity (which may be equated to nucleotide diversity) was particularly high (π = 5.8) within the BV population in comparison with other local populations (Tab. 2). This may be a consequence of the predominant current patterns offshore of Maputo Bay and the Delagoa Bight (Fig. 1) (Lutjeharms 2006), which circulate bay waters in a clockwise eddy. These local currents may inhibit the spread of propagules out of this breeding population. Pairwise F_{ST} values indicated that BV was significantly distinct from other populations sampled. Further, other *A. austera* populations in the study appear to differ in growth pattern from those at BV (Fig. 3). Once BV was removed from analyses, further tests of variability demonstrated that the remaining *A. austera* populations were panmictic. Haplotypes were shared widely between populations and there was no evidence of reciprocal monophyly (Fig. 2). This was similar to results of previous regional work on *Pocillopora verrucosa,* which showed local panmixia (Ridgway *et al.* 2001).

Local *A. austera* populations are expected to rely on migrants from the north to maintain levels of genetic diversity, in a similar fashion to that reported for *Pocillopora verrucosa* (Ridgway *et al.* 2008). This may be confirmed by a larger scale analysis which includes more populations from this region. With longer-lived coral species, such as *P. daedalea* (in comparison to *A. austera)*, it is possible that there may be enough migration to sustain a high level of genetic homogeneity throughout a larger area, as was found for *Plesiastrea versipora* in the western Pacific (Rodriguez-Lanetty and Hoegh-Guldberg 2002). However, although *P. daedalea* populations are well differentiated (γst = 0.29), the ITS region is notoriously difficult to work with (Lam and Morton 2003; Vollmer and Palumbi 2004, Mangubhai and Souter 2007) and regions with such high recombination rates may not yield conclusive results at this scale. Thus, other than to encourage further research using more conclusive molecular markers and larger population sizes, little may be drawn from analyses of the ITS region of *P. daedalea* in this study.

Southward movement the water is very gradual ($0.05ms^{-1}$ south) in the north of the study area, but the current is fast flowing from between Inhaca and Maputaland ($1.5ms^{-1}$ south, Lutjeharms 2006). These currents do not necessarily preclude movement of propagules in a northerly direction in nearshore water movement. East African reefs are thus unlikely to be connected to the same extent amongst all genera considering the different reproductive strategies amongst local scleractinians (Mangubhai and Harrison 2006). Population genetic studies of species with very different life strategies, such as *A. austera* and *P. daedalea,* will help to shed light on this issue, especially if fine-scale variability within and between populations is measured. Future research in the SWIO region would benefit from employing molecular markers which display the high resolution necessary to detect this variability.

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Connectivity of Queen conch, *Strombus gigas*, populations from Mexico

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Abstract

Despite active conservation measures, slow recovery of Queen conch (*Strombus gigas)* is a growing concern in the Caribbean. Conventional expectations presume that *S. gigas* populations are largely connected, but we present results of veliger larval drift and survival from the Yucatan peninsula that suggest otherwise. Spatial patterns of observed and simulated larval stages during the reproductive season revealed segregation of the Mexican populations, with high levels of larval retention on Campeche Bank contrasted to variable larval transport along the Mexican Caribbean coast into the Yucatan Current, and eventually into the Loop Current. Consequently, the probability that *S. gigas* larvae originating from the Mexican Caribbean settle to Alacranes Reef is null. In addition, the Alacranes *S. gigas* population is not source to Florida, while a small fraction of larvae produced north of the Mexican Caribbean coast periodically reaches the Florida Keys (*ca.* 1-12%) and Cuba (0.1-4%), while very few individuals reach the NW Bahamas (0-0.5%). Although this long-distance dispersal may not be sufficient to replenish the downstream populations, gene flow could prevent differentiation of the Florida Keys and Mexican Caribbean Queen conch populations. This study constitutes a first step in understanding Queen conch metapopulation structure and calls for more local actions for the recovery of Mexican populations.

Keywords: Queen conch; larva; population connectivity; biophysical modeling; isolation

Introduction

The Queen conch, *Strombus gigas* (Mollusca, Gastropoda: Strombidae), has been a significant marine resource for the Caribbean since pre-Columbian times. Since the 80's, commercial catch has increased in response to the international market. Consequently, several stocks have been reduced to levels where the population can no longer recover. Queen conch was included in 1992 in the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora, and in 1994 and was added to the International Union for the Conservation of the Nature's (IUCN) Red List. Following the pattern of other Caribbean countries, a decline in abundance in the 80's led to the closure of the Yucatan fishery (Pérez-Pérez and Aldana-Aranda 2000). Presently, there is a temporal ban during peak spawning season, from March to October, a harvest quota for Chinchorro Bank and Cozumel, and a minimum capture size. In spite of these measures, for the most part the distribution of Queen conch in the area remains confined to marine protected areas. Given similar conservation measures for the area, one might expect a uniform response from the population, but this does not appear to be case, as evidenced by discrepancies between Alacranes Reef on Campeche Bank, and Chinchorro Bank situated on the Caribbean coast (Aldana-Aranda et al. 2003). The population of *S. gigas* in Alacranes Reef was almost depleted after 1988 and recent studies still report relatively low abundances there (Pérez- Pérez and Aldana-Aranda 2000). In contrast, despite lack of enforcement, the population situated at Chinchorro Bank has remained relatively stable. These observations suggest that Alacranes Reef may be disconnected from other populations along the Caribbean coast. Effective management of these populations could be greatly enhanced by better enforcement but also by understanding of larval movements. According to a theory developed by Stoner (1997), currents carrying larvae from the Yucatan Peninsula to the Bahamas and Florida could also provide larval drift between the Mexican Caribbean coast and the North Yucatan Peninsula. Studies of the abundance of larvae have been carried out in Florida and the Bahamas (De Jesús-Navarrete and Aldana-Aranda 2000) with the intention to protect the larval sources of *S. gigas*. This study examines Queen conch larval transport with the goal of testing Stoner's null hypothesis that geographically separated *S. gigas* populations are linked. Our major objectives are to describe patterns of *S. gigas* larvae around the Yucatan Peninsula and

their potential transport to Florida, the Bahamas, and Cuba, and identify likely larval sources in the Yucatan Peninsula. We use both field studies and biophysical modeling coupling currents with *S. gigas*' life history traits. We use field observations to corroborate the numerical model predictions, and then probabilistic simulations to estimate larval linkages.

Study Area

The study region was limited to test the null hypothesis that Queen conchs in Mexico form a single

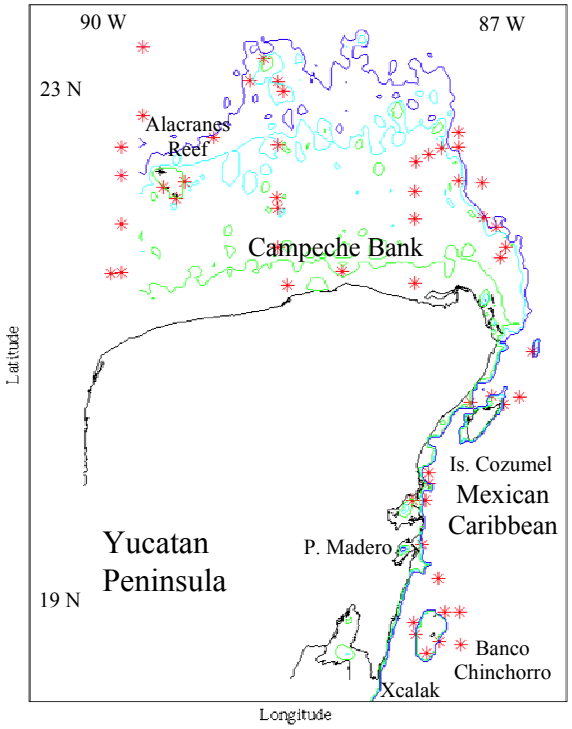

Figure 1. Sampling sites (red stars) in the Yucatan Peninsula during 2001. Green, cyan, and blue lines represent isobaths 20, 60, 100 m, respectively. The Alacranes lagoonal reef is located at the edge of the 60 m isobath on the west of the Campeche Bank, sole home of the Queen Conch on the Bank.

population. The Yucatan Peninsula has two distinct marine regions: (1) the Mexican Caribbean (MC) coast with a narrow shelf, bordered by back reef lagoons, shallow bays and banks; (2) the Campeche Bank with a wide shelf and a single small bank, the Alcranes Reef (AR, Fig. 1). The Caribbean Current veers northwestward as it impinges the margin of the Yucatan peninsula and becomes the Yucatan Current, which enters the Gulf of Mexico as the Loop Current and exits through the Florida Straits as the Florida Current. In addition, a large cyclone-anticyclone pair south of Cuba feeds occasionally into the Yucatan Current. Transport in the Yucatan channel is characterized by the main surface inflow into the Gulf of Mexico and deep reverse outflows along the Cuban and Mexican sides (Cherubin et al. 2005). On the Mexican side, deep outflow occurs at the level of the cape of the continental margin (22°N, 86.5°W, nearby the group of stations 38-41 in Fig. 1), forming a convergence zone near the surface. On average, the Yucatan Current flow is weaker from November to February and becomes stronger from May to August. Spawning of *S. gigas* occurs from March to October, with 1-6 spawning events per female producing benthic egg masses (Pérez-Pérez y Aldana-Aranda 2000). Veliger larvae become competent on average at 20 days after hatch (dah), but metamorphosis can occur anywhere between 12-60 dah. Similarly plastic, the average pelagic larval duration is 30 d. Veliger are usually found in the upper 5 m of the water column, but are capable of vertical migration.

Material and Methods

Observations

Plankton was sampled at 83 stations during oceanographic surveys of the Marine Secretary from the north of Chuburná Puerto and Cabo Catoche during March, July, and October of 2001. Sea surface temperature and salinity were recorded. Near surface (1m) plankton tows were made with a Bongo net of 0.61 m diameter and 302µm mesh size. Tow durations were 15 min at 1 $m.s^{-1}$ and volume filtered was measured using a calibrated flow-meter. Samples were preserved at sea in a 5% neutral formaldehyde-seawater solution. In the laboratory, samples were sorted using a dissecting microscope (20x). *S. gigas* were identified following Davis et al. (1993). Shell length (SL) was measured with a calibrated ocular micrometer and veligers were divided in two size classes and aged based on laboratory growth rates (Brito-Manzano and Aldana-Aranda 2004): pre-competent larvae < 700 µm SL, ≤ 20 days; competent larvae > 700 µm LS, ≥ 21 days.

Biophysical Model and Simulations

Larval transport was simulated using daily archived velocity fields of the North Atlantic HYbrid Coordinate Ocean Model (HYCOM; 10 m coastal boundary; 1/12° horizontal resolution; http://hycom.rsmas.miami.edu) coupled to a particle tracking algorithm. The code described in (Paris et al. 2007) integrates adult spawning strategies, conch larval traits, and recruitment habitat. Pre-competent particles (or virtual larvae) were passive, but at competency they could sink if their path crossed suitable nursery habitat. However, if the particle reached maximum competency without finding habitat, it was removed from the system. There were to many assumptions related to introducing larval mortality in the model due to lack of data on larval survivorship while egg mass survival tend to change throughout the spawning season (Aldana-Aranda et al. 2003). Thus mortality was not accounted for,

which, on the other hand, tends to overestimate long distance dispersal (Cowen et al. 2000), specifically when pelagic duration is variable (Paris et al. 2007). The habitat data was developed using reef locations provided by the Millennium Coral Reef Mapping Project (http://imars.usf.edu/corals) buffered at 5 km

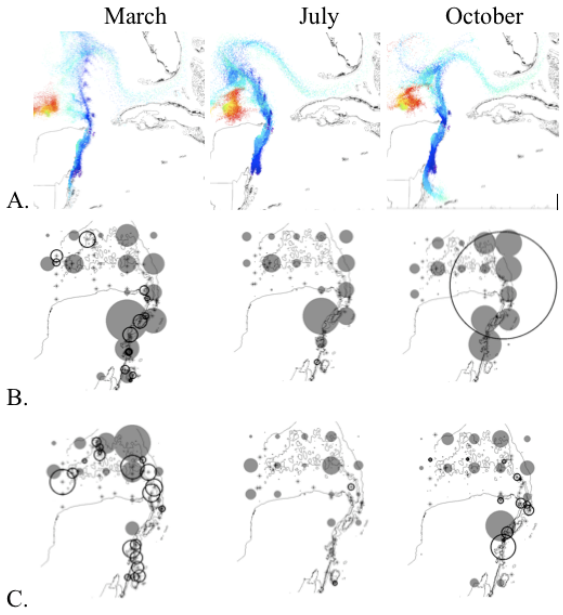

Figure 2. A) Simulated dispersal of *S. gigas* larvae released during the first day of March, July, and October from 2 spawning locations. Color gradients indicate dispersal time from Alacranes Reefs (yellow » red = post-hatch » competent larvae) and along the Mexican Caribbean (blue » cyan). B) Modeled (grey dots) and observed (black circles) distribution patterns of pre-competent (1-20 days old, size < 700 µm) *S. gigas* larvae. C) Modeled and observed patterns of competent larvae (21-40 days old, size > 700 µm). The virtual larvae have a pre-competent, passive period of 20 d and a maximum pelagic duration of 40 d.

and split using a tolerance level of 10 km. A total of 60, 5, 50, and 355 reef polygons shaped the MC, AR, FLK, and Bahamas respectively. In a first scenario (*Model 1*) virtual released during March, July and October from the 83 stations sampled in 2001 were done to validate the biophysical dispersal model by comparison of observed and modeled larval distributions. Particle release at each station was scaled by observed densities, while particle 'age' at release corresponded to the mean developmental stage of the sample. In a second probabilistic scenario (*Model 2*), a series of 1000 particle releases were initiated in the 0-20 m layer with a monthly frequency from February to October at all 65 locations (60 MC, 5 AR) within the Yucatan Peninsula region (Fig, 2A). The source and receiving location for individual particles was recorded to build connectivity matrices and estimate the probability of larval exchange between and among regions.

Results

Spatio-temporal abundance and size of veligers

Observations on the North Yucatan Shelf: during March, 50% of the 31 stations sampled (1-37, Fig. 1) yielded 68 larvae, with a maximum density of 1.8 • 10^{-1} m^{-3} at station 4, about 100 km south of Alacranes Reefs (Fig. 2C). Mean density was 0.28•10^{-1} m^{-3} and size ranged from 520-990 µm (mean 774 µm), with a tendency towards competent larvae. In July, 17% of the 12 sites yielded few pre-competent larvae (i.e. 4 larvae, maximum density 0.07•10^{-1} m^{-3}, mean size 512 µm). In October, 30% of the 17 stations sampled yielded 18 larvae with a maximum density of 0.1• 10^{-1} m^{-3} at station 27 north of Cabo Catoche. Most larvae

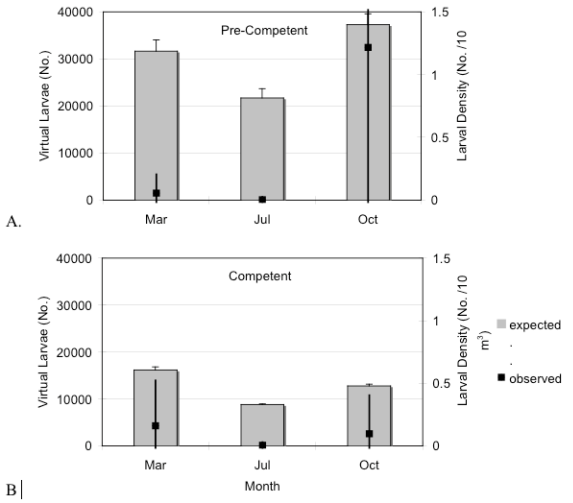

Figure 3. Average temporal trends of pre-competent and competent conch larvae around the Yucatan Peninsula from A) point observations at stations (black squares) and B) modeled distributions (filled grey bars) during one year. Mean and standard deviation are calculated from the stations sampled and the binned distribution (black circles and filled grey filled, respectively, in Fig 2). Note similar trends betwen modeled and real abundances with higher variance in observations (i.e. more spatial patchiness).

were competent with a size range of 670-970 µm (mean 873 µm).

Observations along the MC Coast: during March 2001, 57% of the 30 stations sampled (stations 38-83, Fig. 1) yielded 82 larvae with a maximum density of 0.9•10^{-1} m^{-3} at station 66 (Fig. 2C). Size ranged from 350-990 µm with a tendency towards early stage larvae (mean 645 µm). Mean density was 0.17•10^{-1} m^{-3}. In July 17% of the 18 stations sampled yielded only 6 larvae. Similarly to the North Yucatan Peninsula, mean density was very low (0.01• 10^{-1} m^{-3}) but larvae were competent (mean size 825 µm, range 680-970 µm; Fig. 2C). In October, 60% of the 16 stations sampled yielded the largest number of larvae (n = 1642) with a maximum density of 43.2•10^{-1} m^{-3}

in a single patch of 1402 larvae at station 40, northeast of Holbox Island at the cape where (Fig. 2B). Catches were composed of all stages as sizes ranged from 520-990 µm (mean size 783 µm, mean density $1.93 \cdot 10^{-1}$ m-^{3}).

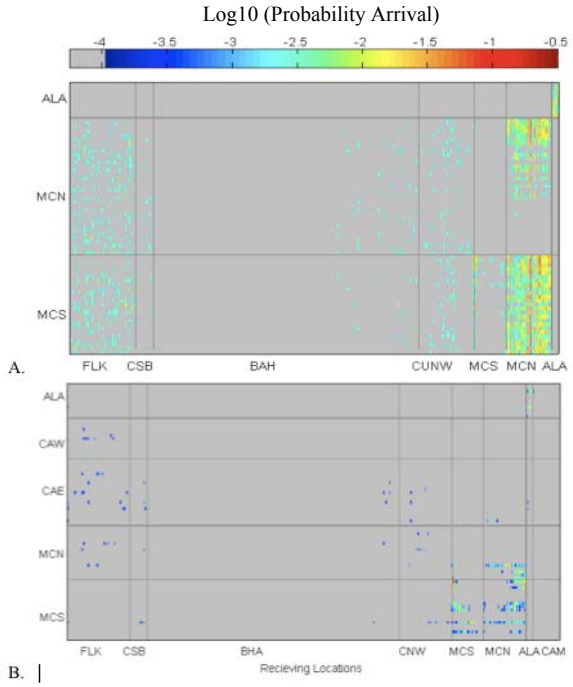

Figure 4. Connectivity matrices between *S. gigas* populations from the Yucatan Peninsula, Florida Keys, Bahamas, and NW Cuba estimated from A) probabilistic scenario (Model 2): homogeneous monthly (Feb-Oct) spawning events from 65 reef locations of the Yucatan and B) predictive scenario (Model 1): scaled production, initializing larval transport from observed distributions of larvae. Each matrix elements is the probability of larvae from source *i* (Y-axis) moving to destination *j* (X-axis) over the reproductive season. Color-code indicates levels of connectivity, grey is no larval exchange. The source and receiving locations are: Alacranes (ALA); Mexican Caribbean S (MCS, Ascension Bay to Chinchorro Bank); Mexican Caribbean N (MCN, Ascension to Contoy); Campeche Bank East & West (CAM, CBE, CBW); Florida Keys (FLK); CaySal Bank (CSB); Bahamas (BAH); NW Cuba (CUNW).

Comparison of observed and modeled distributions

Observations and predictions agree well early in the reproductive season (i.e. March), when early stages are found in both locations with an noticeable separation between the MC the western part of Campeche Bank, near Alacranes Reef (Fig. 2B). More advanced larvae are predominant in the North Yucatan (Fig 2C). This is an indication that both areas are sources. Monthly patterns are also well predicted by the model, with lower densities during July (Fig. 3), when currents along the MC are swifter (Tang et al. 2005). In addition to higher dispersal, atmospheric conditions may have been unfavorable for the larvae. Indeed, veliger are presumably more abundant near the surface during calm seas (Barile et al. 1994) but they sink in turbulent conditions. This would have affected regional-scale sampling. During October, *S. gigas* densities increased in both model and predictions, but observations were more patchy with 1402 larvae caught a single station. This rare catch was composed of a mixture of larvae from 10-30 dah (520-890 µm), indicating that they have been accumulating in that area for more than 10 days. Larval vertical behavior in convergence zones could create large patches of larvae. The model did not predict accumulation of larvae in a small area such as through eddies. Yet, virtual larvae from 20 to 30d old were accumulated along a strong frontal convergence (Fig. 2B). According to the model, this large larval patch could have contributed to recruitment during November in the Dry Tortugas, but not as an unusual larger recruitment peak. When the model is run in a probabilistic mode with a series of temporal releases from all possible source locations (Model 1) rather than predictive (Model 2), the spatial pattern of larval densities is re-established. Thus we are confident that the biophysical model is consistent with the observations and can be used to estimate larval exchanges between regions.

Modeled Dispersal around the Yucatan Peninsula

Larval dispersal from the MC coast is variable and extends south to the northern atolls of the Belize Barrier Reef, and north to the lower FLK (Fig. 2A). On the other hand, dispersal from the North Yucatan is limited and less variable with high retention around AC. In July, higher northward transport of the Yucatan Current in the simulations produced lower larval densities along the eastern edge of the Peninsula (Fig. 2C).

Larval exchange between sub-regions

There is high connectivity within sub-regions and clear isolation of AR (Fig. 4). The MC export a small fraction of total recruits to FLK (12%), 4% to NW Cuba, and <1% to 16 polygons of the 355 polygons representing the Bahamas, none to AR. Most (82%) settlement was local, the southern part of the MCS being a large source to the north (MCN, Fig. 4).

Discussion

The planktonic nature of the Queen conch larvae suggests that ocean currents would quickly cause panmixis over large spatial scales and prevent isolation of populations. However, slow recovery of the Queen conch in the Caribbean, especially in AR and FLK, contradicts this panmixis hypothesis. While genetic studies do not show population structure in the Caribbean (Sobel et al. 1988), this study shows far greater isolation than hypothesized by Stoner (1997). As a consequence, *S. gigas* populations could develop genetic differences that can only be resolved by using fast evolving genes (e.g. Baums et al. 2005). This

study suggests that AR is not a source for the Floridian *S. gigas* populations. The Lower FLK may occasionally receive a small fraction of recruits from the MC (i.e., 1-12%, Fig. 3). The strength of the Yucatan Current as well as the northward intrusion of the Loop Current seems to control the level of exchanges. However, this long distance dispersal may not sustain the FLK populations. In fact, the FLK populations of *S. gigas* have been under a total catch ban since 1985 but have not shown signs of recovery indicating that subsidies are not sufficient to sustain the population. The southern MC appears to be an important source of *S. gigas* larvae and a corridor for populations located on either side. De Jesús-Navarrete and Aldana-Aranda (2000) found a high percentage (89%) of early stages larvae in Chinchorro Bank, and hypothesized that this site is a potentially important source of veliger *S. gigas* to other sites of the Quintana Roo coast, and possibly to Florida. We do not find evidence that Chinchorro Bank contributes recruits to FLK, but it is most probably a source for the MC coast. Indeed, the model indicates that potential source locations of *S. gigas* for the MC are located from Chacala and Tulum to Xcalak, including Chinchorro Bank, and from Puerto Madero and Majahual to Xcalak. However, locations between Puerto Madero and Majahual to Xcalak seed only the northern portion of Quintana Roo. De Jesús-Navarrete and Aldana-Aranda (2000) also indicates that competent larvae were found in Cayo Lobo, south of Chinchorro Bank and assumed that they might have come from distant sources in Belize. Yet, throughout the *S. gigas* reproductive season, alongshore currents can reverse, allowing larval drift from Quintana Roo coast towards the south, including part of the Belize Barrier Reef (Fig. 2A). Detailed larval linkages are worth investigating further to map networks of *S. gigas* populations within the Mesoamerican region.

Risk of species extinction from an unpredictable physical environment and/or strong interactions between species is minimized by metapopulation structure (Harrison and Taylor 1997). This may be the case for the lower Florida Keys population, which is weakly connected with the MC. Indeed, if retention is a common phenomenon for this species, then the exchange rate of veligers would be low enough to fit the metapopulation assumptions (Harrison and Taylor 1997). The latter explains the fact that the Queen conch has a wide distribution of fragmented populations in the Caribbean and yet may maintain genetic continuity. Thus, conservation measures should take into account local networks of sub-populations. Isolated populations, which have a higher risk of extinction, should be protected and managed separately. In summary (1) the populations from the North Yucatan Peninsula and the Mexican Caribbean are segregated, with major isolation of the Alacranes Reef, while spawning entities along the Mexican Caribbean are highly inter-connected; (2) This study does not support Stoner's hypothesis of long distance larval linkages between Queen conch populations in the Caribbean. Simulations reveal a weak connectivity of the Mexican Caribbean with the Lower Florida Keys. Yet, there are no significant exchanges with the Bahamas (i.e., 2-3 order of magnitude lower recruitment levels in < 4% of the Bahamian habitat) and clear isolation of the North Yucatan Peninsula; (3) although the biophysical model reproduces trends in larval densities, there is mismatch in snapshots of spatial distributions. Biological factors such as aggregation in fronts, sinking in turbulence and mortality (Paris et al. 2007) must play a role in determining absolute levels of settlement.

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Genetic variation in two morphotypes of *Porites panamensis* from the Gulf of California, Mexico

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Abstract. Genetic variation was analyzed to determine whether two morphotypes, a massive and columnar form, of *Porites panamensis* found together in the Gulf of California (GC) were genetically differentiated. Levels of genetic variation were similar between morphotypes and no fixed alleles were detected between them. Levels of sexual reproduction were high, as indicated by ratios N_G:N 0.76-1.00 and G_O:G_E 0.71-1.00 for both morphotypes. Analysis of Molecular Variation (AMOVA) indicated the greatest significant genetic variation within populations (97.85%) and among populations within morphotypes levels (2.63%), but not among morphotypes (-0.47%, p = 0.6826). Mean significant F_{ST} values for columnar (F_{ST} = 0.024) and massive (F_{ST} = 0.043) suggest that both morphotypes had moderate genetic structure within their populations. The number of migrants per generation (N_em) showed differences within morphotype populations (columnar 4.65-31 and massive 2.65-9.75). The lower genetic differentiation among morphotypes indicates that it is likely that they frequently interbreed. However, we observed a genetic differentiation among the populations of the morphotypes of *P. panamensis* in the north and central part of the GC, while in the south of the GC the populations of both the morphotypes are more similar and may constitute a population that is more genetically homogeneous. North-central coral communities from the GC are characterized by extreme temperature and nutrients conditions and adaptation to this stress environment may be reflected in our genetic data.

Key words: Species complex, genetic structure, Scleractinian coral, population genetics.

Introduction

The genus *Porites* Link 1807 has a cosmopolitan distribution and comprises around 60 of the world's main reef-building species (Veron 2000). It has proved difficult to efficiently delimit the taxonomic position of several species of this genus because of their considerable morphological diversity, resulting in overlapping of characters among taxa (Weil 1992; Garthwaite et al. 1994; Veron 2000). In the Gulf of California (GC), a complex of species presents this problem, involving the nominal taxon *Porites panamensis* Verrill 1866. This coral exhibits four colonial morphologies that were described originally as different species: massive (*P. panamensis, sensu stricto*), encrusting (*P. californica*), columnar (*P. nodulosa*) and branching form (*P. sverdrupi*). These nominal species were considered lately to be ecotypes of different depths and synonyms due the high morphological variation in the genus (Squires 1959; Wells 1983). However, morphometric and genetic studies indicate that at least the ramified form (*P. sverdrupi*) is a valid and endemic species of the GC (López-Forment 2003; López-Pérez et al. 2003; Forsman et al. 2006). The columnar and massive morphologies of *P. panamensis* have presented slight differences in the number of pali of their corallites (Ketchum and Reyes-Bonilla 2001). In field, differences between these forms were found in their susceptibility to algal colonization, which could mean that the columnar morphotype has a survival advantage over the massive (Paz-García and Reyes-Bonilla 2006). In vertical distribution, the massive morphology is from 1 to 30 m of depth and the columnar one is present from 1 to 5 m (López-Pérez et al. 2003; Paz-García and Reyes-Bonilla 2006). In addition, the massive morpotype is distributed from the GC to Colombia, while the columnar one is observed only inside the GC and Revillagigedo's islands (Ketchum and Reyes-Bonilla 2001; Paz-García and Reyes-Bonilla 2006). It is still unidentified if these differences between morphotypes are due the morphological plasticity or if they correspond to different species. Allozyme electrophoresis studies have been showed useful to distinguish species

boundaries in the genus *Porites* (Weil 1992; Garthwaite et al. 1994) and to assess the contribution of reproductive mode (sexual vs asexual) within the populations (Ng and Morton 2003; Nishikawa and Sakai 2005). Furthermore, the presence of different species or the morphology itself by means fragmentation could show differences between morphotype populations along the GC. The aims of this study were to (1) determine if the columnar and massive morphotypes of *P. panamensis* were genetically differentiated to clarify the taxonomic position of this group of morphospecies, (2) describe the genetic variation between the morphotype populations, (3) determine the contribution of reproductive mode (sexual vs asexual) within morphotype populations of *P. panamensis* in the GC.

Material and Methods

Field Work. In August of 2004, we collected 55 and 58 coral fragments for columnar and massive morphotypes, respectively, using SCUBA. The specimens were collected in one to three coral communities (1-9 m of depth and over an area of 400 m^2) within each collection area. The collection areas were in the GC, Mexico (Fig. 1): Bahía de Los Ángeles (BLA), Isla San Marcos (ISM), Bahía Concepción (BCO) and Sourth of Bahía de La Paz (BLP). The coral fragments were frozen in liquid nitrogen and transported to the Biochemistry Lab from the Centro de Investigaciones Biológicas del Noroeste at La Paz, BCS, Mexico where they were stored at –80°C.

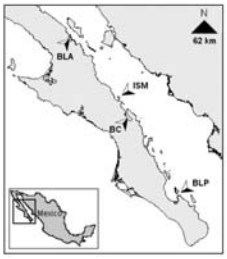

Figure 1: Map of Gulf of California showing four collection populations. **BLA** Bahía de Los Ángeles, **ISM** Isla San Marcos, **BCO** Bahía Concepción, **BLP** South of Bahía de La Paz.

Allozyme Electrophoresis. One or two ml of blastate was obtained with a gun of air pressure from SCUBA tank and rinsed with seawater. The blastate still contained zooxanthellae and although these possess own genetic material, in previous studies it has been observed that not affect the genetic analysis by allozyme on corals (Stoddart 1983; Willis and Ayre 1985). However, the microalgae symbionts were isolated of the host tissue by centrifugation to minimize the possibility of contamination (Stoddart 1983; Weil 1992). One milliliter of blastate was centrifugated at 2600 *g* for 4 min at 4°C. The supernatant was placed in vials and mixed with 40 µl of Stoddart's buffer modification (Stoddart 1983; Weil 1992). Zooxanthellae enzymes extracted from coral tissue did not appear on our gels. The concentration of total proteins from each sample was analyzed by Bradford's method (Bradford 1976) and 25 µg of sample was used for the analysis. Four enzyme systems were used: *Mdh* (E.C.1.1.1.37), *Leu-leu*, *Leu-tyr* and Leu-gly-gly peptidases (E.C.3.4.11). Allozyme analysis was carried out using the method of Polyacrilamide Gel Electrophoresis (PAGE) by discontinuous gel system in native conditions (Laemmli 1970; Manchenko 1994).

Statistic analysis. Genetic variability (Swofford and Selander 1981), $N_G{:}N$, and $G_O{:}G_E$ (see Table 1 legend) were calculated for each population of both morphotypes (Stoddart and Taylor 1988). Unbiased genetic distances (D) were used for cluster analysis (Nei 1978). We performed an analysis of molecular variance (AMOVA) to compare genetic similarity between the morphotype populations, P-values were calculated from a random permutation test with 16 000 replicates (Excoffier et al. 1992). The level of genetic heterogeneity and gene flow among morphotypes were estimated by F_{ST} and the average number of migrants per generation (*Nem*), respectively. We calculated pairwise F_{ST} estimates between each pair of populations (within and among population morphotypes). F_{ST} were tested for difference from zero permuting (10 000 replicates) alleles between samples with exact G-test (Goudet *et al*. 1996), as implemented in FSTAT v. 2.8 (Goudet 1995). We applied a sequential Bonferroni correction to reduce the chance of type I errors (Rice 1989).

Results

No fixed alleles were detected between morphotypes. The mean number of alleles per locus at each location ranged from 1.9 to 2.4 for the columnar morphotype and from 2.1 to 2.3 for the massive one (Table 1). The observed heterozygosities were slightly higher that the expected under Hardy–Weinberg equilibrium in all populations, ranging from 0.331 to 0.486 for columnar morphotype and 0.331 to 0.529 for massive morphotype (Table 1). The ratios $N_G{:}N$ (0.76-1.00) and $G_O{:}G_E$ (0.71-1.00) for both morphotypes indicate a high rate of sexual reproduction and that this strategy is the most important in the maintenance of their populations in the GC (Table 1). AMOVA indicated the greatest significant genetic variation within populations (97.85%, $p<0.001$) and among populations within morphotypes levels (2.63%, $p<0.001$), but not between morphotypes (-0.47%, $p = 0.6826$). The values of Nei´s (1978) unbiased genetic

distances within morphotype populations, ranged from 0 to 0.020 and 0 to 0.050 for columnar and massive morphotypes, respectively (Table 2). The values of genetic distance between samples of different morphotypes ranged from 0 to 0.047. Cluster analysis showed three groups by geographical proximity in the GC: one group included both northern morphotype populations, a second group formed by central massive populations, and center-south populations of the columnar with the southern population of the massive morphotype as another cluster (Fig. 2).

Table 1. Genetic variability and relative contribution of the sexual and asexual reproduction of populations of columnar and massive morphotypes of *P. panamensis* from the GC. *N* number of individual colonies at each population, N_G number of unique genotypes observed at each population, G_O and G_E observed and expected genotypic diversity, respectively. Population abbreviation as in Fig. 1. Standard errors in parentheses.

Morphotype	**Columnar**				**Massive**			
Population	*BLP*	*BCO*	*ISM*	*BLA*	*BLP*	*BCO*	*ISM*	*BLA*
N	13	14	14	14	13	14	17	14
Mean no. of alleles/locus	1.9 (0.1)	2.0 (0.0)	2.4 (0.2)	2.2 (0.1)	2.3 (0.2)	2.2 (0.1)	2.3 (0.2)	2.1 (0.2)
Observed heterozigosity	0.331 (0.05)	0.486 (0.04)	0.429 (0.02)	0.457 (0.05)	0.331 (0.05)	0.507 (0.03)	0.529 (0.03)	0.429 (0.07)
Expected heterozigosity	0.273 (0.04)	0.374 (0.02)	0.395 (0.02)	0.392 (0.04)	0.301 (0.03)	0.424 (0.02)	0.412 (0.02)	0.333 (0.05)
N_G:*N*	0.769	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G_O:G_E	0.719	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 2. Nei´s unbiased genetic distance (below diagonal) and F_{ST} values (above diagonal) for the morphotypes of *Porites panamensis* from the GC. Population abbreviations as in Figure 1. *p<0.05, ***p<0.01 after Bonferroni correction.

	Population		1	2	3	4	5	6	7	8
1	Col	BLP	———	0.008	**0.023***	**0.051****	0.009	0.032	**0.066****	**0.045***
2	Col	BC	0.000	———	0.001	**0.031***	0.000	0.000	0.031	0.018
3	Col	ISM	0.005	0.000	———	0.032	0.013	0.000	0.022	**0.030***
4	Col	BLA	0.020	0.014	0.018	———	**0.053****	**0.034***	**0.071****	0.018
5	Mas	BLP	0.001	0.000	0.003	0.025	———	0.037	**0.070****	0.036
6	Mas	BC	0.007	0.000	0.000	0.019	0.014	———	0.000	0.025
7	Mas	ISM	0.029	0.014	0.011	0.047	0.036	0.000	———	**0.086****
8	Mas	BLA	0.015	0.003	0.013	0.005	0.014	0.008	0.050	———

Mean significant F_{ST} values after Bonferroni correction were observed for columnar (F_{ST}=0.024, p<0.01) and massive (F_{ST}=0.043, p<0.01) morphotypes. Pairwise F_{ST} estimates revealed no significant differences between the populations of both morphotypes in the same location, but significant differences were observed between the populations of BLA from both morphotypes and the rest of the GC (Table 2). N_em showed differences between morphotypes; the columnar presented higher values (4.65-31) among its populations in comparison with the massive (2.65-9.75). N_em between populations of different morphotypes ranged from 3.27 to 27.52.

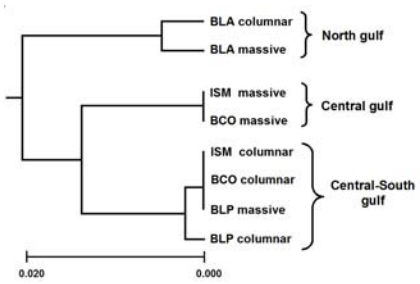

Figure 2: UPGMA dendrogram showing the populations of columnar and massive morphotypes of *P. panamensis* in the GC based on Nei´s (1978) unbiased genetic distance.

Discussion

No fixed alleles were detected between columnar and massive morphotypes of *P. panamensis* and AMOVA indicated that the variation was not significantly partitioned between morphotypes (-0.47, p=0.6826). In addition, the Nei´s (1978) unbiased genetic distance between morphotypes was 0.0009. These data suggest that both morphotypes interbreed frequently and are likely to be the same species. However, slight genetic differences were observed between morphotype populations. The columnar morphotype had lower genetic differentiation within populations (D <0.020) than the massive one (D <0.050). The values of genetic distance within populations of the columnar morphotype are similar to those reported in literature for populations with slight genetic differentiation, such as *Platygyra sinensis* (D <0.008; Ng and Morton 2003) and *Mydedium elephantotus* (D <0.015; Yu J-K et al. 1999). The values of genetic distance obtained within populations of the massive morphotype and among the populations of the morphotypes are more similar to the distances reported for those species that possess genetic structure in their populations such as *Goniastrea aspera* (D <0.040, Nishikawa and Sakai 2005) and *Pocillopora damicornis* (D < 0.066; Adjeroud and Tsuchiya 1999).

The dendrogram of Nei´s unbiased genetic distance showed three groups of populations clustering mostly by geographical vicinity (Fig. 2). This pattern showed the subdivision exists among the populations of the morphotypes of *P. panamensis* in the north part and central of the GC, while in the south of the GC the populations of both the morphotypes are more similar and may constitute a population that is more genetically homogeneous. Mean significant F_{ST} values observed in both morphotypes (F_{ST} > 0.024, p<0.01) suggest that those have a genetic structure within their populations along the GC. Genetic structure in both morphotypes can be explained by the

brooding larvae of *P. panamensis* that are recruited to some few meters of the parental colonies (Glynn and Ault 2000). Pairwise F_{ST} estimates revealed no significant differences between the populations of both morphotypes in the same location, but significant differences were observed between the populations of BLA from both morphotypes and the rest of the GC (Table 2). Studies carried out with diverse taxa have suggested the north area of the GC is a different biogeographic region; it has based on the pattern of distribution of species, salinity differences, temperature, tide, high eutrophic conditions, to the population subdivision and the reduction of the genetic flow in marine invertebrates and fishes (De la Rosa-Vélez et al. 2000; Riginos and Nachman 2001, Halfar et al. 2004). This difference may be due to combined effects of biogeography, geographical distances, and habitat discontinuity that could result in different evolutionary histories among populations (De la Rosa-Vélez et al. 2000; Riginos and Nachman 2001). In addition, the coral community of *P. panamensis* from Bahía de Los Angeles is characterized by temperatures extremes (ranging from 14°C to 30°C), lower penetration of light (-9m), the highest values average in chlorophyll in coral communities of high latitudes (2.2 mg Chl a/m), high content of phosphates (1.8 µmol/l) and nitrates (9.9 µmol/l) that affect the growth and coral development (Halfar et al. 2005). This community can be adapted to temperature stress and nutrients (Halfar et al. 2005), and may be reflected in our genetic data. The morphotypes showed differences in N_em in populations with separation of 750 km; the columnar form presents three times higher number of migrants within its populations in its maximum values in comparison with the massive one (4.65-31 vs 2.65-9.75). Similar values of migrants per generation have been estimated in populations with separation of 1700 km for broadcast spawners (4.8-24.8) and brooding species (1.4-19; Ayre and Hughes 2004). However, gene flow among populations is affected by diverse factors such as the reproductive mode of the studied species, overlapping generations, dispersal ability, habitat discontinuity, available space for recruitment, oceanographic conditions and isolation by distance (Stoddart 1983; Ayre and Hughes 2004).

Previous studies of allozyme electrophoresis in coral species have revealed populations that reproduce mainly sexually, asexually or with a mix of reproductive mode (Stoddart 1983; Yu et al. 1999; Nishikawa and Sakai 2005). Our data show that asexual reproduction (e.g. from fragmentation, budding, or fission) had little influence on the maintenance of the populations of *P. panamensis*, which is consistent with ecological studies (Glynn et al. 1994; Reyes-Bonilla and Calderón-Aguilera 1994). High ratios of N_G:N = 1 and G_O:G_E > 0.719 suggest that sexual reproduction in both morphotypes of *P. panamensis* is the most important means of maintaining their populations in the GC. These results are consistent with life history characteristics of the columnar morphotype in BLP, which can brood larvae over the whole year (Mora-Perez 2005). In addition, the population BLP of the columnar morphotype presented some clonal multilocus genotypes (Table 1). This suggests that this population is occasionally subject to reproduction via fragmentation, which is consistent with its colonial morphology and the incidence of hurricanes in the south of the GC. The absence of colonies with clonal multilocus genotypes in the massive morphotype is also consistent with its colonial morphology that is less likely to fragment. The generally high frequencies of unique multilocus genotypes in most populations of both morphotypes of *P. panamensis* may be due to diverse habitats and oceanographic conditions along the GC (López-Pérez et al. 2003; Halfar et al. 2005; Paz-García and Reyes-Bonilla 2006), to the high frequency of sexual reproduction and recruitment during the whole year (Mora-Perez 2005), or to the type and the frequency of moderate environmental disturbances (i.e. ENSO events and hurricanes) that may favor genotypic diversity in sexually reproducing coral communities (Coffroth and Lasker 1998). Our data suggest a higher sexual reproduction rate for massive morphotypes than columnar, however there are no reproductive studies of the massive morphotype in the GC. Studies carried out in Jalisco and Oaxaca showed that the massive morphotype reproduces during the warm months (May to September; Vizcaíno-Ochoa 2003; Rodríguez-Troncoso 2006). If this occurs in the GC, differences in the reproductive season among morphotypes (columnar whole-year vs massive warm season reproduction) could explain in part the genetic differences observed between the morphotype populations.

The observed differences among morphotype populations in the GC may be due to intrinsic factors (e.g. differences in expulsion times of sperm gametes and larvae, selective recruitment, differences in larvae dispersion), as it has been found in the members of the *Montastraea annularis* complex (Weil and Knowlton 1994; Knowlton et al. 1997). In addition, certain host genotypes from massive and columnar morphotypes were associated specifically with a particular *Symbiodinium* type and depth strongly influenced the frequency of occurrence of particular symbionts in populations of both morphotypes individuals (Paz-García et al. 2009). The possibility of host-symbiont co-evolution in *Porites panamensis* morphotypes is important due high or low light-

adapted symbiont may directly affect the differential success of larvae settling in deep or shallow environments. Further work should address to explain if the latitudinal genetic differentiation and genetic structure morphotypes depend on vertical distribution pattern, clade simbiont and/or intrinsic factors.

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Genetic structure of the massive coral *Porites panamensis* (Anthozoa: Scleractinia) from the Mexican Pacific

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Abstract. Genetic structure was studied in the brooding coral *Porites panamensis* along of the Mexican Pacific (MP). We collected in three locations inside of the Gulf of California, two zones at the entrance of the Gulf, and one location at south of MP. Exclusive genotypes in two of six allozyme loci were observed in northern (LGG-1^{DE} and LGG-1^{EE}) and southern (EST-1^{AA} and EST-1^{AB}) populations. All populations presented significant deficits of heterozygotes. These deficits could be for different recruitment and temporal events of larvae expulsion along the MP, high local recruitment and inbreeding by limited dispersion of larvae, and different mortality events by natural disturbances. Cluster analysis of genetic distance showed three groups by geographic proximity: the populations from inside of the GC, two populations from the entrance of the gulf, and the southern population of MP. AMOVA indicated a significant differentiation among the three groups (11.93%, $p = 0.016$). Mean significant F_{ST} value ($F_{ST} = 0.104$, $p < 0.01$) reveled a genetic structure. The oceanic patterns coupled with restricted dispersion of this brooding coral species could be the principal factor that generating the genetic structure observed.

Key words: Population structure, Scleractinian coral, brooding coral, population genetics

Introduction

Porites panamensis is the most abundant coral in the central and north of the Gulf of California and is observed in most coral communities from the Mexican Pacific (Glynn and Ault, 2000; Reyes-Bonilla, 2003). This coral has been reported from 31°N in Mexico to Isla Gorgona (3°N), Colombia (Glynn and Ault, 2000; López-Pérez *et al.*, 2003). *P. panamensis* is a gonochoric species with internal fertilization and colonies begin to reproduce at 5 cm^2 (Glynn *et al.*, 1994). Asexual reproduction by fragmentation has not been observed as important roll in their recruitment (Glynn *et al.*, 1994; Reyes-Bonilla and Calderón-Aguilera, 1994; Paz-García *et al.*, 2009b). Their larvae settle a few meters from the parental colonies (Glynn *et al.*, 1994) and perhaps this reflects a limited capacity of dispersion (Glynn and Ault, 2000). Substrate cover of this coral species in the Gulf of California varies between 1 to 7% of the substrate; in some areas it exhibits a dense covering and in other this totally absent (Reyes-Bonilla and Calderón-Aguilera, 1994; Halfar *et al.*, 2005). ENSO 1982-83 event produced massive mortalities in coral communities of Central America and this species disappeared almost completely in this region (Guzmán *et al.*, 1987; Weil, 1992; Glynn *et al.*, 1994). In Mexico, the damage in the coral communities of this species was lower, and the reproduction and local recruitment of this species only diminished during the ENSO events (Reyes-Bonilla and Calderón-Aguilera, 1994; Medina-Rosas *et al.*, 2005; López-Pérez *et al.*, 2007). Our aim was to determine the genetic structure of the massive coral *P. panamensis* along of the Mexican Pacific (MP).

Material and Methods

Field Work. Collections were conducted from 2004 to 2006 in different areas of the Mexican Pacific (Fig. 1): Bahia de Los Angeles (BLA), Isla San Marcos (ISM), Bahia Concepcion (BCO) and South of Bahia de La Paz (BLP), Punta Arenas (PAV), Isla Redonda (IRD) and La Entrega (LET). The specimens were colleted in shallow coral communities (1-5 m). The coral fragments were frozen in liquid nitrogen, transported to the laboratory, and subsequently stored at –80°C.

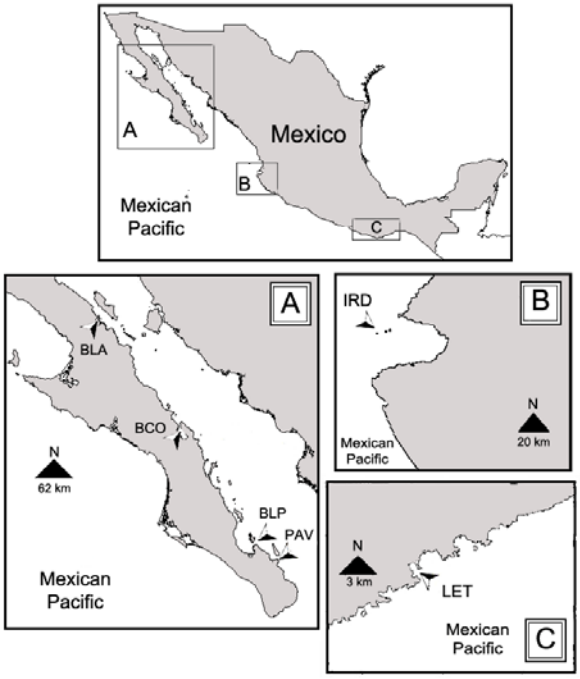

Figure 1: Map of Mexican Pacific showing the collection populations. **BLA** Bahia de Los Angeles, **BCO** Bahia Concepcion, **BLP** South of Bahia de La Paz, **PAV** Punta Arenas, **IRD** Isla Redonda, **LET** La Entrega.

Allozyme Electrophoresis. 3g of superficial tissue-skeleton coral were placed in two vials with 0.7 ml of Stoddart's buffer (Stoddart, 1983; Weil, 1992). The vials were placed in a cold bath of a sonicator and two milliliters of blastate was centrifugated at 2600 *g* for 10 min at 4°C. The resulting supernatant was placed in vials and concentration of total proteins for each sample was determined by Bradford's method (Bradford, 1976) and 25 µg was used for the analysis of each enzyme system (Paz-García *et al.*, 2009b). Allozyme analysis was carried out using the method of Polyacrilamide Gel Electrophoresis (PAGE) by discontinuous gel system in native conditions (Laemmli, 1970; Manchenko, 1994). Four enzyme systems were used: leucine-glycil-glycil peptidase (LGG, E.C.3.4.11.1), malic enzyme (ME, E.C.1.1.1.40), glutamate dehydrogenase (GDH, E.C.1.4.1.3) and esterase (EST, E.C. 3.1.1.1).

Statistical analysis. Genetic variability was calculated for each population using BIOSYS-1 software package (Swofford and Selander, 1981). Unbiased genetic distances (Nei, 1978) were used for cluster analysis, as implemented in TFPGA (Miller, 1997). We performed an analysis of molecular variance (AMOVA) to compare genetic similarity among three groups: I) populations from the GC, II) entrance of GC, and III) the population from the south of MP. P-values were calculated from a random permutation test with 16 000 replicates (Excoffier *et al.*, 1992). The level of genetic heterogeneity was estimated by F_{ST}. We calculated pairwise F_{ST} estimates between each pair of populations. F_{ST} were tested for difference from zero permuting (10 000 replicates) alleles between samples with exact G-test (Goudet *et al.*, 1996), as implemented in FSTAT v. 2.8 (Goudet, 1995). We applied a sequential Bonferroni correction to reduce the chance of type I errors (Rice, 1989).

Results

Five loci were detected using four enzyme systems. We observed exclusive genotypes from the most northern (BLA: LGG-1^{DE} and LGG-1^{EE}) and southern (LET: EST-1^{AA} and EST-1^{AB}) populations (Anexus I). The allelic diversity at each location ranged from 2.4 to 2.8 (Fig. 2a). The observed heterozygosities were lower that the expected under Hardy–Weinberg equilibrium in all populations, ranging from 0.106 to 0.260 (Fig. 2b).

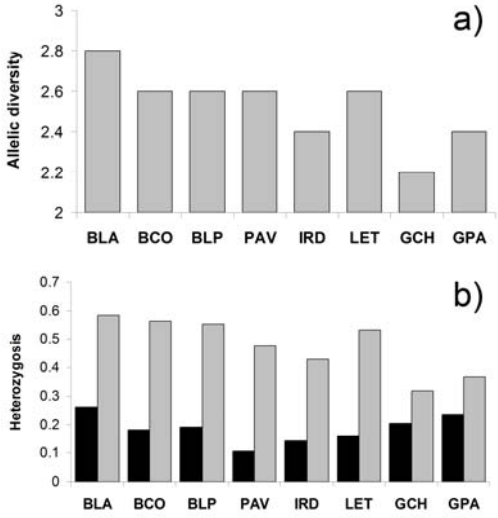

Figure 2. a) Allelic diversity. b) Heterozigosis. Observed and Expected Heterozigosis, black and gray, respectively. Population abbreviation as in Fig. 1. **GCH** Gulf of Chiriqui and **GPA** Gulf of Panama (data from Weil, 1992).

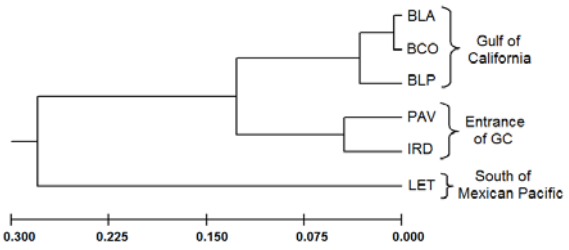

Figure 3. UPGMA dendrogram based on Nei´s (1978) unbiased genetic distance. Population abbreviation as in Fig. 1.

Cluster analysis based on Nei's (1978) unbiased genetic distance, showed three groups by geographical proximity: (I) the populations from inside of the GC, (II) two populations from the entrance of the Gulf, and (III) the southern population of MP as other cluster (Fig. 3). AMOVA indicated a significant differentiation among the three groups (11.93%, p = 0.016), among populations within groups (3.38%, p = 0.036), and within populations (84.68%, p < 0.001). Mean significant F_{ST} value (F_{ST}=0.104, p < 0.01) was observed for the massive populations of *P. panamensis*. Pairwise F_{ST} estimates revealed significant differences among populations along the MP, except the populations from inside of the GC (Table 1).

Table 1. F_{ST} values (below diagonal) and p values (above diagonal) for the massive coral *Porites panamensis* from the MP. Population abbreviations as in Figure 1. F_{ST} were tested for difference from zero permuting (10 000 replicates) alleles between samples with exact G-test (Goudet *et al.*, 1996). NS = not significant, * p < 0.001 after Bonferroni correction.

	Population	1	2	3	4	5	6
1	*BLA*	———	0.014^{NS}	0.010^{NS}	0.000*	0.000*	0.000*
2	*BCO*	0.013	———	0.000*	0.000*	0.000*	0.000*
3	*BLP*	0.016	0.024	———	0.000*	0.000*	0.000*
4	*PAV*	0.048	0.071	0.062	———	0.000*	0.000*
5	*BB*	0.070	0.082	0.103	0.031	———	0.000*
6	*OAX*	0.096	0.095	0.118	0.124	0.118	———

Discussion

Allelic diversity in two populations of *P. panamensis* from the Panamanian Pacific showed values ranged from 2.2 to 2.4 alleles per locus (Fig. 2a; Weil, 1992). For the present work, most populations of *P. panamensis* from the MP presented slight higher values (2.4-2.8). The observed heterozygosities, ranged from 0.205 to 0.236 in two populations of *P. panamensis* from Panama (Fig. 2b; Weil, 1992). All populations of *P. panamensis* from the MP were slight higher that this (Fig. 2). During the 1982-83 ENSO event, populations of *P. panamensis* from the Panamanian Pacific suffered severe mortalities that caused their disappearance almost completely (Guzmán *et al.*, 1987; Glynn *et al.*, 1994). In Mexico, the recruitment of this species diminished during the ENSO events (Reyes-Bonilla and Calderón-Aguilera, 1994), but contrary to the reefs of Central America, this species suffered lower mortalities, it has continued reproducing and their larvae have recruited locally (Medina-Rosas *et al.*, 2005; Mora-Pérez, 2005; López-Pérez *et al.*, 2007). Genetic variation observed among the populations of *P. panamensis* from the Mexican and the Panamanian Pacific, may be due to the difference in the mortality presented in both regions, since it has been mentioned that the heterozygosities diminishes fast when the size population is reduced (Gillespie, 1998).

A deficit of heterozygous is characteristic of several coral species and several explanations for such heterozygosity deficits have been proposed (Ayre *et al.*, 1997; Ayre y Hugnes, 2004). Heterozygousity deficits observed in populations of *P. panamensis* along of the MP could be due to different recruitment events of cohorts and mixes of adult colonies from diverse coral communities (Medina-Rosas *et al.*, 2005; López-Pérez *et al.*, 2007), different temporal events along the MP when larvae expulsion takes place (Vizcaíno-Ochoa, 2003; Mora-Perez, 2005; Rodríguez-Troncoso, 2006; Paz-García *et al.*, 2009b), high local recruitment and inbreeding by limited dispersion of larvae (Glynn y Ault, 2000); and different mortality events by natural disturbances (Reyes-Bonilla *et al.*, 2002).

The dendrogram based on Nei's (1978) unbiased genetic distance showed three groups by geographic proximity (Fig. 3). Our data supports the similarity among the populations inside of the GC and the differentiation between the entrance of the GC (PAV y IRD), and the southern population of the MP (LET). Same pattern of population subdivision in the MP was observed in *Pocillopora damicornis* (Chávez-Romo *et al.*, 2008). These results suggest that the inside populations of the GC may be a group more homogeneous genetically, while the populations of the entrance of the GC (PAV y IRD) and southern of MP (LET) present a genetic differentiation that may be due at the high frequency of natural phenomenon (e.g. hurricanes, upwelling zones and mortality by ENSO events).

Genetic structure among populations of *P. panamensis* in the MP was supported by mean AMOVA (11.93%, p = 0.016) and significant F_{ST} value (F_{ST}=0.104, p < 0.01). Previous studies in the GC and MP have found population subdivision in different marine invertebrates and fishes groups (De la Rosa-Vélez *et al.*, 2000; Riginos y Nachman, 2001; Valles-Jimenez *et al.*, 2005). Genetic structure was reported in other coral species along of the California cost (*Balanophyllia elegans*, mean F_{ST} = 0.195; Hellberg, 1996), and along the MP in the species *Pocillopora damicornis* (F_{ST} 0.153, Chávez-Romo *et al.*, 2008) and *Pavona gigantea* (Φ_{ST} 0.10-0.20; Saavedra-Sotelo, 2007). Several factors may help generate population genetic subdivision in the MP, including biogeography, geographic distance, habitat discontinuities, current direction, and differences in the environmental conditions (e.g. temperature, tide, eutrophic conditions by upwelling zones), could result in different evolutionary histories among populations (De la Rosa-Vélez *et al.*, 2000; Riginos and Nachman, 2001; Halfar *et al.*, 2005; Valles-Jimenez *et al.*, 2005). We suggest other factors that could be generating the pattern observed: differences in

reproductive seasons among coral communities along the MP (Paz-García *et al.*, 2009b; Chávez-Romo *et al.*, 2008), selection of resistance genotypes due at the differential blanching and mortality events (Reyes-Bonilla *et al.*, 2002), the presence of different symbiont clades in one same host species and the possibility of different combination between host and symbiont along the MP, could be represent different opportunities to support low light conditions and high level of environment stress (LaJeunesse *et al.*, 2007a, b; Paz-García *et al.*, 2009a, b), and the existence of long sand barriers and mangroves communities that may limit the larval dispersion between coral communities (Glynn y Ault, 2000). Also, the oceanic patterns coupled with restricted dispersion of brooding coral *P. panamensis* could be the principal factor that is generating the genetic structure observed on the populations in the MP. However, detailed studies in ecology, reproduction and genetics are necessary to understand better the relationships between the coral populations in the MP.

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Annex I. Allele frequency of six populations of *P. panamensis* from the Mexican Pacific. Population abbreviation as in Fig. 1. *N* number of samples analyzed at each population.

Población	*BLA*	*BCO*	*BLP*	*PAV*	*IRD*	*LET*
LOCI						
ME-1						
N	20	20	20	34	20	25
A	0.675	0.625	0.625	0.868	0.775	0.360
B	0.325	0.375	0.375	0.132	0.225	0.640
GDH-1						
N	20	13	20	34	20	25
A	0.450	0.462	0.400	0.706	0.650	0.720
B	0.550	0.538	0.600	0.294	0.350	0.280
GDH-2						
N	20	19	20	34	20	25
A	0.250	0.210	0.300	0.353	0.200	0.240
B	0.450	0.474	0.500	0.412	0.600	0.480
C	0.300	0.316	0.200	0.235	0.200	0.280
EST-1						
N	20	20	20	34	20	25
A	—	—	—	—	—	0.660
B	0.475	0.375	0.425	0.750	0.900	0.340
C	0.525	0.625	0.575	0.250	0.100	—
LGG-1						
N	20	20	20	34	19	25
A	0.150	0.400	0.075	0.059	0.237	0.260
B	0.300	0.350	0.125	0.338	0.605	0.480
C	0.125	0.200	0.500	0.382	0.158	0.080
D	0.300	0.050	0.300	0.221	—	0.180
E	0.125	—	—	—	—	—

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How do swimming ability and behaviour affect the dispersal of coral larvae

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Abstract. Most marine larvae have a free-swimming phase before settling. This phase could be important for retention, dispersal, and connectivity. It was thought that coral larvae swimming capabilities were so poor that currents advected them. Nowadays it is accepted that coral larvae have some ability to control their position. This study describes, from laboratory observations, the swimming capabilities and changes in swimming behaviour with age of *Montastraea annularis* and *M. faveolata* larvae, and how these might promote or hinder dispersal. Three larvae from each species were observed and their position, swimming velocity, and behaviour were recorded every five minutes. GLM ANOVA was used to determine the effects of age on velocity and depth. To determine the effect of age on larvae behaviour a binomial logistic regression analysis was performed. The results show the capability of larvae to alternate swimming pattern, velocity and depth which enables them to explore suitable substrata for settlement. This capability can be used to control their position in the water column, and reduce or increase dispersal. Additionally, we found, for the first time 1) swimming velocity and depth varied with age for *M. annularis*; and, 2) the probability of settlement increased with age for both species.

Key words: swimming behaviour, larval age, larval dispersal, *Montastraea*

Introduction

Almost all marine invertebrates have free-swimming larvae (Young et al. 2002). Until recently it was widely assumed that while in the plankton, larvae were advected by currents as passive particles (e.g. Jackson 1986), and that the duration of planktonic larval stages were correlated to the dispersal distance (Strathmann 1985; Shanks et al. 2003a). Nowadays it is known that larvae can swim or change their behaviour avoiding advection (Byers, Pringle 2006). Thus, larvae can disperse further than predicted (Leis, Carson-Ewart 2003), or settle very close to their parents reducing genetic dispersal (Cowen et al. 2006). Swimming ability can also be crucial on searching for a suitable substratum on which to settle (Kingsford et al. 2002). The alteration in swimming behaviour during active searching for settlement sites is often in response to specific cues (e.g. Krug, Zimmer 2000; Baird et al. 2003; Hadfield, Koehl 2004).

Swimming capabilities vary among marine larvae. Reef fishes and decapod crustaceans are capable swimmers, overcoming in some cases local currents (Zeldis 1985; Luckenbach, Orth 1992; Leis, Carson-Ewart 2003). Coral larvae and cyphonautes larvae are examples of poor swimmers (e.g. Abelson 1997; Shanks et al. 2003b; Strathmann, Grünbaum 2006). Even weak currents move faster than coral larvae (Brooke, Young 2005). However, both capable and poor swimmers can avoid being adversely advected by currents by modifying their behaviour and controlling their position in the water column (Fuchs et al. 2004; Brooke, Young 2005). For coral larvae this behaviour appears to be mediated by the detection of water pressure (Stake, Sammarco 2003).

Larval life histories influence swimming behaviour and later settlement processes. In many cases, larvae can extend their time in the plankton but this has increased associated costs (Pechenik 1990). For lecithotrophic larvae these costs may be depletion of energy reserves and larval senescence (Pechenik 1990). Surprisingly little is known about changes in swimming behaviour as coral larvae age, and how these changes could affect dispersal. Mundy and Babcock (1998) found that five out of six species of coral larvae responded either to light quantity or quality. Additionally, they observed that competent larvae were more positive geotactic than non-competent larvae. In 2005, Brooke and Young reported that the larvae of ahermatypic coral *Oculina varicosa*, when fully developed, swam towards the surface in a spiral fashion.

Larvae of benthic organisms spend most of their time in the water column either searching for food

and/or a suitable substratum on which to settle. Studying the swimming behaviour is important to understand population ecology processes such as settlement, recruitment, and population composition (Mumby 1999). Before we can understand how dispersal is influenced by the swimming behaviour of the invertebrate larvae, is essential to determine the swimming abilities of planktonic larvae throughout the entire larval period. Using coral larvae reared in the laboratory, the aim of this study was to document changes in larval swimming behaviour (categories, velocity and depth) with age, and how these changes could promote or hinder dispersal from parental reefs. We hypothesised that changes in swimming behaviour will occur with age and that these changes would enhance settlement. We studied coral larvae from *Montastraea annularis* and *M. faveolata*, two of the most important reef builder species in the Caribbean.

Material and Methods

Gamete bundles of both *M. annularis* and *M. faveolata* were collected during spawning nights in 2004 and 2005 at San Andres Island (Western Caribbean, Colombia). After spawning, gamete bundles were transported to the laboratory where fertilisation was performed. Cross fertilisation was designed to avoid both self-fertilisation and fertilisation between different species. Self-fertilisation was avoided by separating eggs and sperm after gamete bundles were collected. Egg/sperm were separated using a 60 μm plankton mesh. The eggs were rinsed several times with fresh filtered and 90 ^{o}C heated seawater. Fertilisation between different species was avoided by keeping gamete bundles, eggs and sperm from each species in labelled vials. Coral larvae were reared following Pizarro et al. (2006).

When larvae were fully developed (Young et al. 2002) observations on swimming behaviour were performed. Larvae were placed into a cylinder (1 m high x 20 cm diameter) of transparent acrylic (3 mm thick) filled with sterilised seawater (Pizarro et al. 2006). A metric ruler was fitted to the exterior of the cylinder. Three larvae were observed daily, each for one hour, and vertical position (depth), swimming velocity (cm s^{-1}), and the type of swimming behaviour they were performing. These data were recorded every five minutes. Swimming categories were named after the movement the larvae performed (table 1).

To determine the effects of the age of the larvae on the swimming velocity and swimming depth, a separate repeated measures GLM ANOVA was conducted for each response variable. Because the response data were not normally distributed and not transformable a value of $\alpha = 0.001$ was taken as the level of significance (Underwood 1997). In both cases the data were not spherical, therefore Greenhouse-Geiser adjusted F values were used. For both analyses the within subject factor was age and the response was either velocity or depth.

To examine the effect of age on the behaviour of the larvae a binomial logistic regression analysis was used to predict the probability of settlement behaviour as a function of larval age. All upward swimming categories were associated with transport behaviour, while all downward swimming categories were associated with settlement behaviour. For the remaining swimming categories transport behaviour and settlement behaviour were determined by a combination of the observed swimming category of the larvae and the depth at which the swimming category was taking place. For example, horizontal swimming in the first 10 cm of the water column was recorded as transport behaviour, whereas, if it occurred any deeper it was recorded as settlement behaviour.

Results

Twelve different swimming categories were identified for *Montastraea faveolata* larvae, and eleven of the twelve were observed for *M. annularis* larvae (Table 1). The frequency varied within categories and between species (Table 1). While for *M. annularis* the most frequent category was swimming down (28.6 %), for *M. faveolata* it was snake swimming (38.91 %).

Table 1. Name and frequency (percentage of the total number of observations) for swimming and settlement behaviour (percentage for each swimming category) of the swimming categories identified for *Montastraea annularis* (*Mann*; n = 1287) and *M. faveolata* (*Mfav*; n = 897) larvae.

	Swimming (%)		Settlement (%)	
Swimming category	*Mann*	*Mfav*	*Mann*	*Mfav*
Circles	0.00	2.12	0.00	36.84
Up	23.62	9.92	0.00	0.00
Spiral – up	0.31	2.23	0.00	0.00
Spiral	10.18	0.78	1.54	28.57
Horizontal	1.17	1.00	40.00	55.57
Snake	10.26	38.91[a]	0.77	0.00
Spiral – up & down	0.23	0.33	53.33	33.33
Still	22.22	11.04	3.31	25.25
Up and down	0.39	5.24	60.00	62.16
Spiral – down	0.78	3.57	100.00	100.00
Down	28.59[a]	12.26	100.00	100.00
Exploring	2.25	12.60	100.00	100.00

[a] denotes the most frequent swimming category for each species

The frequency of each swimming category did not vary with the larvae age (Fig. 1). However the most commonly observed categories appeared to have a periodicity in both species (e. g. see first panel in Fig. 1 – swimming up category). Nevertheless, the snake swimming category for *M. annularis* was the only one

that seemed to change considerably with the age of the larvae (Fig. 1). For this particular category, the frequency increased with the age of the larvae until day 9, after which it was rarely observed.

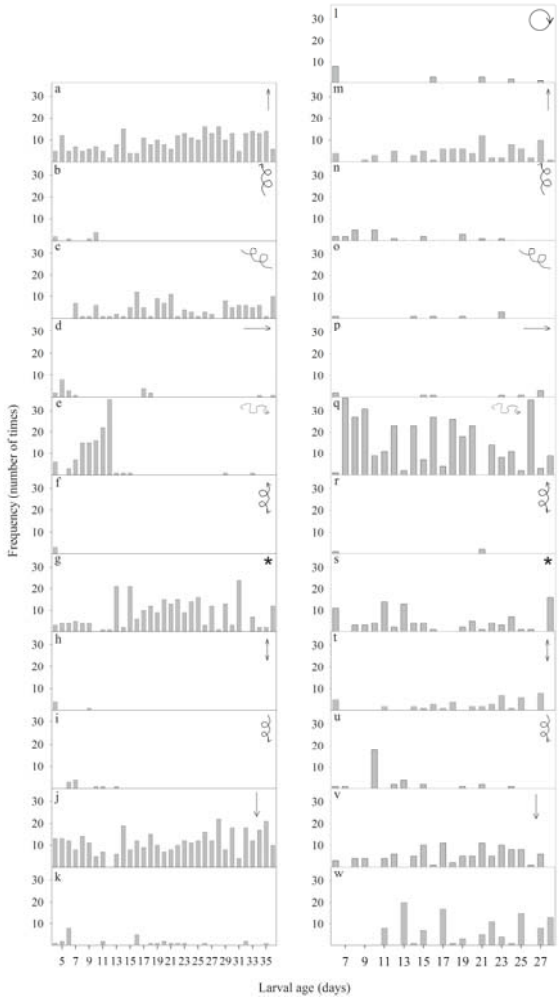

Figure 1. Frequency of each swimming category as a function of the age of the larvae for *Montrastraea annularis* (a-k) and *M. faveolata* (l-w). Pictograms identify behavioural category and are arranged in the same order as in Table 1.

Recorded swimming velocities (cm s^{-1}) for *M. annularis* were 0.01-1.4 and, 0.01-1.6 for *M. faveolata*. For *M. annularis* larvae swimming velocity and depth varied with age (repeated measures GLM SS_{vel} = 2.35; df_{vel} = 10.74; F_{vel} = 6.44; P_{vel} < 0.001; SS_{depth} = 158892.15; df_{depth} = 9.63; F_{depth} = 7.82; P_{depth} < 0.001). The larvae started to explore the water column from the first day they were fully developed. A plot of mean depth vs. day indicated that there was a periodicity in the variation of depth. Simple time series analysis using moving averages of between 2 – 4 d suggested that this periodicity was ~ 3 d (Fig. 2 a-c).

Swimming velocities of *M. faveolata* larvae did not change with age (repeated measures GLM SS = 7.30; df = 1.178; F = 2.00; P = 0.162), however depth of the larvae behaviour did change with age (repeated measures GLM SS = 488857.45; df = 5.126; F = 26.32; P < 0.001). 1 d to 5 d old larvae remained mostly at the surface and larvae between 6 d and 23 d old moved from the surface to deeper in the water column. A periodicity in the variation of the depth of ~ 3 d (Fig. 2 d-e) was also found for this species when applying a simple time series analysis.

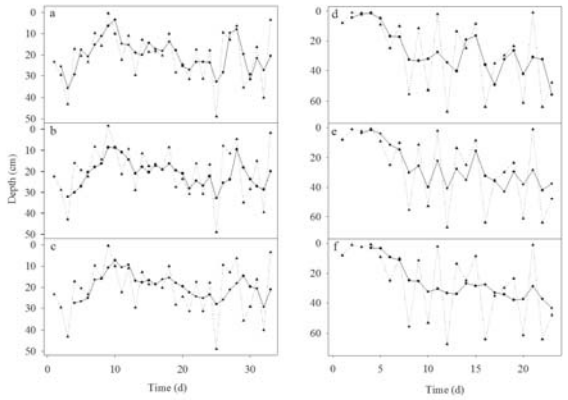

Figure 2. Mean depth of *Montastraea annularis* (a-c) and *M. faveolata* (d-f) larvae as a function of time (dotted line; *M. annularis* n= 33 and *M. faveolata* n= 23), and results of the moving averages for time series analysis (continuous line) for two (a, d), three (b, e) and four (c, f) days. Periodicity was determined by peak coincidence rather than amplitude

The probability of occurrence of settlement behaviour when compared to transport behaviour was 1.01 and 1.09 times higher with each day's increase in age for *M. annularis* and *M. faveolata* respectively (*M. annularis*: $P_{\text{settlement behaviour}} = 1/(1 + e^{-(-0.26 + 0.11 \text{ Age})})$, Odds ratio = 1.02, P < 0.001, Log-likelihood = G = 11.38, DF = 1, P < 0.001; *M. faveolata:* $P_{\text{settlement behaviour}} = 1/(1 + e^{-(-1.31 + 0.15 \text{ Age})})$, Odds ratio = 1.09, P < 0.001, Log-likelihood = G = 62.83, DF = 1, P < 0.001).

Discussion

This study shows for the first time that coral larvae have a range of different swimming behaviours and that 1) swimming velocity and depth of the larvae of *M. annularis* varied with age; and, 2) the probability of settlement-linked behaviour increased with age for both *M. annularis* and *M. faveolata*. The role of different swimming behaviours in pre-settlement processes of corals has only been studied somewhat superficially (e.g. Carlon, Olson 1993; Raimondi, Morse 2000). Furthermore, swimming behaviour and the relationship to transport and settlement have not been previously recognised (e.g. Morse et al. 1988;

Gross et al. 1992; Abelson 1997; Krug, Zimmer 2000; Raimondi, Morse 2000; Miller, Mundy 2003). It is common knowledge that most coral larvae are active swimmers (see Harrison, Wallace 1990). This study has clearly shown the capability of larvae to vary swimming pattern, velocity and depth which then enables them to explore suitable substrata for settlement. This capability is also likely to contribute to dispersal away from natal reefs by controlling their position in the water column (see Harrison, Wallace 1990), and may determine adult distributions on reefs (Raimondi, Morse 2000).

At least four (spiral, snake, still and circles) of the 12 swimming categories described in this study could be considered as contributors to the dispersal of larvae in coastal waters (Porch 1998). This suggests that the remaining categories of *Montastraea* spp. larvae (spiral up, spiral down, spiral up and down, down, and up and down) could be important for retention of larvae within natal reefs (Codling et al. 2004). In contrast, if larvae enter oceanic waters, then active swimming could play a role in maintaining depth to ensure maximum dispersion. Such modification of swimming behaviour has been associated with the capacity to control a vertical position in the water column, rather than as a dispersal factor (Raimondi, Morse 2000; Bassim, Sammarco 2003; Krug, Zimmer 2004). In this regard, the observations on the frequency of the swimming categories as a function of time for each species suggest that 1) *M. annularis* larvae would control their vertical position most of the time; and, 2) *M. faveolata* would be affected the most by superficial currents. To determine whether the swimming categories of both *M. annularis* and *M. faveolata* larvae are important in maintaining position in the water column near their natal reefs or in the ocean waters to be dispersed, it is necessary to develop local hydrodynamic models (Mumby 1999). These models would predict if embryos and pre-competent larvae are retained within the natal reefs or carried away from them. However, if larval development and swimming patterns in natural environments are similar to those observed in this study, it is feasible that both self-recruitment and dispersal occur (Sammarco 1996).

The changes in depth with age for both *Montastraea annularis* and *M. faveolata* could be an indication of an increased necessity to search for a suitable substratum on which to settle. These findings are similar to those described by Vermeij et al. (2006) for *M. faveolata*. In their study larval behaviour changed from phototactic to geotactic with age, however, they assessed the vertical movement of the larvae only in standard Petri dishes (∅ 9 cm) with approximately 1.5 cm depth. For some other invertebrates, larval distribution in the water column varies according to the larval development stage (Thiébaut et al. 1998; Krug, Zimmer 2004). Krug (2004) observed that swimming speed in trochophore larvae increased as they developed. Of interest is the periodicity in depth observed for both *Montastraea* species, suggesting a vertical migration in the water column. As a non-feeding larvae (Vermeij et al. 2006), this migration would not be related with/to the daily vertical feeding migration observed in plankton (Pearre 2003). Vermeij et al. (2006) also observed a vertical migration in *M. faveolata* larvae. The migration seemed to be a daily light/life cycle with higher movement at night. Nevertheless, their results have to be carefully analysed due to the use of shallow Petri dishes. Indeed, Marta-Almeida et al. (2006) demonstrated that larvae that do not show diel vertical migrations were affected by currents and upwelling leading to offshore transport. We propose that in *Montastraea* spp. vertical migration is a strategy to enhance both detection of suitable substrata for settlement and also increases the dispersal away from unsuitable substrata (Krug, Zimmer 2004; Ríos-Jara 2005; Marta-Almeida et al. 2006).

In lecithotrophic or non-feeding larvae, energy reserves decrease with larval age (Wendt 1996). For lecithotrophic gregarious organisms a desperate larva hypothesis was proposed in the 50s (Knight-Jones (1951, 1953) and Wilson (1953) reviewed in (Toonen, Pawlik 2001)). This hypothesis states that larvae become less discriminatory in their settlement choice when energetic resources decrease and occurs mostly in gregarious organisms, such as bryozoans and ascidians (e.g. Wendt 1996; Marshall, Keough 2003), and in some non-gregarious gastropods (e.g. Botello, Krug 2006). In all these cases larval metamorphosis decreased with time, and even when larvae could respond to cues and initiate metamorphosis older larvae were unable to complete (e.g. Wendt 1996; Toonen, Pawlik 2001; Marshall, Keough 2003; Botello, Krug 2006). This decrease in energy may influence coral larvae velocity and swimming patterns ultimately affecting population dynamics. The observed changes in swimming velocity and swimming patterns with larvae age in this study suggest that the 'desperate larvae' hypothesis could be applicable to both *M. annularis* and *M. faveolata* species. The results suggest that larvae differing in ontogeny can display different behaviours that could affect potential dispersal. Further studies on larval swimming behaviour would help to elucidate the role of these activities on larval retention and dispersion, as well as the importance for population dynamics.

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Variation in coral recruitment on Fijian reefs

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Abstract. Recruitment patterns of scleractinian and milleporan corals were investigated at Suva Harbour, Great Astrolabe Reef, and Taveuni. The lowest recruitment rate occurred at a site in Suva Harbour while the highest recruitment rate occurred on a reef periodically disturbed by high rates of sedimentation. Recruitment was >7 times greater in summer than winter with the greatest seasonal variation occurring in Acroporidae. Pocilloporidae, Poritidae, Faviidae and unidentified recruits had higher recruitment rates in deeper water while Acroporidae recruited primarily to shallow waters. The number of recruits from broadcast spawning acroporids was high, which was probably related to the high frequency of occurrence of the family. More than half (52%) of all recruits were acroporids.. Recruitment was unexpectedly high at a site with periodically high sedimentation levels. Seawater temperatures recorded during the study fell within the normal range for the area.

Key words: Acroporidae, planulae, Pocilloporidae, Poritidae, larva

Introduction

Fiji has one of the largest coral reef systems in the SW Pacific, with >1000 reefs. Although Fiji pos-sesses hundreds of kilometers of coral reefs that are an important source of food (Quinn & Kalangi 1998) and revenue for many people (Quinn & Davis 1998), little is known about coral recruitment processes on the reefs. Many of the ecological studies of corals in Fiji have been limited to species lists (Gawell & Seeto 1982) or surveys of bleached corals (Cummings et al. 2002). While recruitment patterns of juvenile corals are important to the overall community structure of coral reefs (Harrison & Wallace 1990), there have been no studies that examined the variation in recruitment of corals in Fiji.

This study looked at inter annual, seasonal, and geo-graphic variation in coral recruitment over two years and compared the results with other similar studies. Suspended sediment and subsurface temperature were recorded to document two factors that were considered likely to influence settlement.

Material and Methods

Survey sites

Suva Harbour (SH)

Suva Harbour contains the main port for the capital city of Fiji, Suva (Fig. 1). Two sampling sites were chosen in SH (18°07'S; 178°21'E). One was within the lagoon adjacent to the Suva Channel (SC) and the other, Fish Patch (FP), on the outer reef slope adjacent to the harbor entrance. The Suva Channel reef is near the main port in polluted water (Naidu & Morrison 1994, Davis et al. 1998), FP is in less pol-luted water, but has experienced *Acanthaster planci* infestation (Zann et al. 1990, pers. observation).

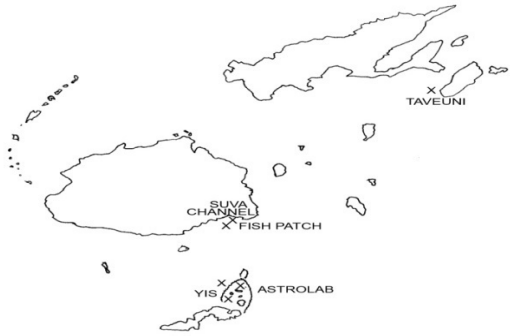

Figure 1. Sampling locations around Fiji.

Great Astrolabe Reef (GAR)

The Great Astrolabe Reef (8°45'S.; 178° 30' E) is an atoll situated 70km south of Viti Levu with a typical depth of 20m. The total human population of the villages on all the 13 islands within the reef is ~1000 people. There were three sampling sites at GAR. One was adjacent to one of the islands, Yanuyanu-i-sau (YIS) (18° 47'S; 178°30'E). YIS is a small, uninhab-ited island, <13.5 ha (Naqasima et al. 1992), that contained seven goats in 1996. The goats, put there years ago by people from the neighboring island, have eaten all of the leafy vegetation to a height of ~1.5m. Consequently, the island is bare of ground vegetation and during heavy rain from December to April the soil washes down the steep slopes onto the fringing reefs.

Taveuni (TA)

Taveuni (16°59'S.; 179°53'E.) (Fig. 1) is an island 200km north of Viti Levu. Except for a few small plantations and resorts, the island lacked any major commercial development.

Physical parameters

Suspended sediment traps 35cm long were constructed from 5.2cm (2") diameter PVC pipes. Paired traps were deployed at all sample sites. The traps were collected periodically between May 1996 and April 1998. Animals, shells, and macro detrital material were removed and the remaining material was dried at 60°C for 24 hrs. The net weight was cal-culated as mg cm^{-2} day^{-1} representing the mean value of each pair of traps.

A Hugrun underwater temperature recorder (UTR) with an accuracy of $\pm$0.05°C monitored subsurface seawater temperatures hourly at GAR from 2 October 1997 to 12 December 1998.

Coral recruitment

Benthic recruitment arrays were constructed using PVC pipe each with four terracotta tiles as described in Quinn and Kojis (2003, 2006). Arrays were deployed at two depths around FP (15m, 25m), one depth in SC (15m), and two depths at TA (15m, 25m). Arrays were placed at three sites in GAR: off the south western outer wall (15m), on a lagoon patch reef (5m) 100m from Dravuni Island, and on a fring-ing reef near YIS (5m, 15m). The arrays were installed in late March / early April 1996. Tiles were replaced in October / November 1996 ("winter 1996"), March / April 1997 ("summer 1997"), September / October 1997 ("winter 1997") and in April 1998 ("summer 1998") and collected about six months later. Numbers of coral recruits on the top, bottom, and edges were counted (standardized to number recruits m^{-2}) and identified to family where possible.

Factors such as orientation of the substratum (Birkeland et al. 1981, Wallace 1985), depth (Babcock 1988); availability of larvae (Harriott & Fisk 1987); interorganismal competition (Harrison & Wallace 1990), browsing herbivores, and dispersal patterns (Sammarco & Andrews 1988), affect recruitment and consequently community structure. The first two factors were standardized in the experiment.

Results

Physical Parameters

Suspended sediment traps were sampled a total of 106 times. The sedimentation rate at each sampling time ranged from <0.1 mg cm^{-2} day^{-1} at GAR from July to October 1996 to 104.9 mg cm^{-2} day^{-1} at YIS from December to April 1997. All of the sites recorded the highest sedimentation rate from December to April. The mean sedimentation rate was highest at YIS and was nearly twice the levels at FP 15m and SC, sites with the next highest sedimentation rates, and 7.5 times higher than the rate recorded from nearby YIS. TA had the lowest sedimentation rate. Sedimentation was most variable within the GAR lagoon sites.

The subsurface sea temperature (S^3T) at Great Astrolabe Reef ranged from 23.6°C in October 1997 to 28.8°C in December 1998 (Fig. 2). The mean S^3T was 26.2°C (CV=4.3%; N=10503). On 15 January 1998, a cyclone passed within 100km of the site and the S^3T dropped 1.1°C within one hour. The mean S^3T during summer 1997 was 26.7°C (CV=4.5%), winter 1998 was 25.7 °C (CV =3.7%) a difference of 0.9 °C.

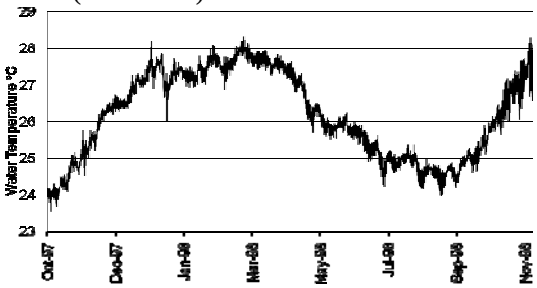

Figure 2: Subsurface seawater temperature at Great Astrolabe Reef

Coral recruitment

A total of 1628 scleractinian and 12 milleporan recruits settled on 72 tiles. The lowest recruitment was at SC (5 recruits m^{-2}, winter 1996) and the high-est at YIS (1749 recruits m^{-2}, summer 1997). Owing to the recruitment in summer 1997, YIS had the greatest total overall recruitment (3624 recruits m^{-2}). As expected the lowest total overall recruitment rate was at SC ($\bar{x}$=51 $\pm$SD 29 recruits $m^{-2}yr^{-1}$) and the highest recruitment rate was at YIS ($\bar{x}$=1812 $\pm$ S.D. 1275 recruits m^{-2} yr^{-1}). FP, 15m, had the next greatest recruitment. The shallow TA site had the third greatest recruitment. Except for the very low recruitment at SH, there was high mean recruitment at the sites (>323 recruits m^{-2} yr^{-1}).

Acroporidae dominated recruitment (52.2%), followed by Pocilloporidae (30.3%), others (13.0%), Poritidae (3.4%), Milleporidae (0.6%) and Faviidae (0.5%). There was a very large recruitment of Acroporidae at YIS in the summer of 1997 represent-ing nearly 30% of the total recruits. This was about double the amount that settled the following summer.

Recruitment rates for the different families varied with depth. Over 55% of the Acroporidae and 67% of the unidentified recruits recruited to the shallowest depth (5m) (Fig. 2). Most Pocilloporidae, Poritidae, Faviidae and others settled in deeper waters. Poritidae recruitment increased with increasing depth. Milleporidae almost exclusively settled at 15m. The rate of recruitment declined with increasing depth at most sites (Fig. 2): 5m - 271 recruits m^{-2}, 15m - 177 recruits m^{-2}, 25m - 142 recruits m^{-2}.

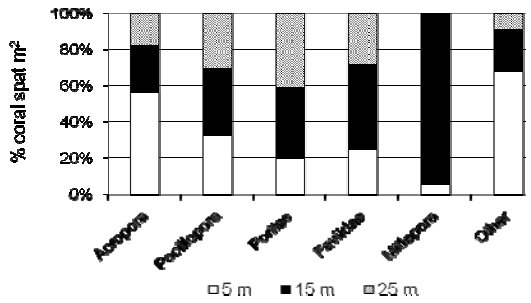

Figure 2: Percentage of coral family recruitment by depth

About six times as many coral spat recruited during the summer ($\bar{x}$=570; N=16; SD=519) compared to winter ($\bar{x}$=93; N =16; SD=101). A paired *t*-test was used to compare mean total corals for each site, there was a very highly significant difference in the rate of recruitment between seasons (*P*=0.0027; N=16). Recruitment was greater in the summer at every site (Fig. 3). The greatest difference was at YIS where recruitment was >10 greater in the summer. In contrast, the difference in the recruitment rate between summer and winter at TA was <10%.

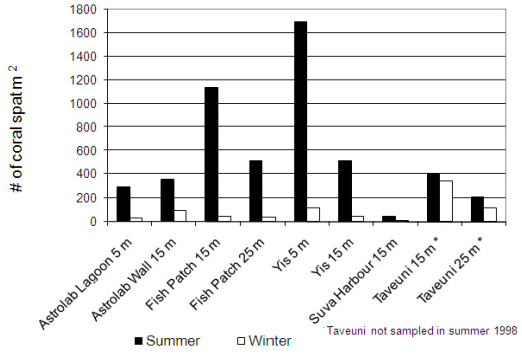

Figure 3: Total number of recruits m^{-2} by seasons and sites

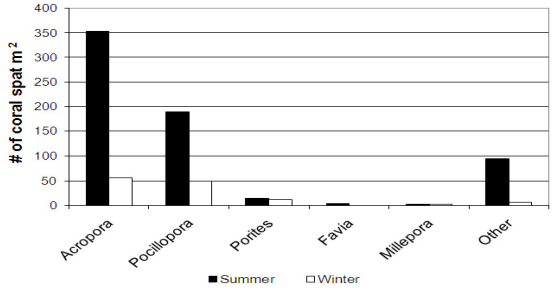

Figure 4: Number of recruits m^2 by family and season

The highest number of recruits were from the families Acroporidae and Pocilloporidae. Their recruitment rate was 7.1 and 3.9 times higher, respectively, in the summer than winter (Fig. 4). Using pooled 15m data a two way ANOVA was performed on the factors years and sites. The location factor was significant (P = 0.027; df = 4), but year was not (P = 0.161; df = 1).

The site with the greatest seasonal difference in Acroporidae recruitment (*t*=2.68; *P*=0.02, S) was YIS (Fig. 5). Seasonal recruitment differences for the Pocilloporidae (*t*=3.71; *P*=0.004; VS) were the great-est at FP (Fig. 6). Seasonal recruitment of Poritidae was variable (*t*=0.21; *P*>0.05; NS) with two sites recording more recruitment during winter than in summer (Fig. 7). The recruitment rates of both Mil-leporidae and Faviidae were much lower than other families. Faviidae recruited only in summer. Uniden-tified recruits, termed "other", settled seven times more frequently in summer than in winter (Fig. 8).

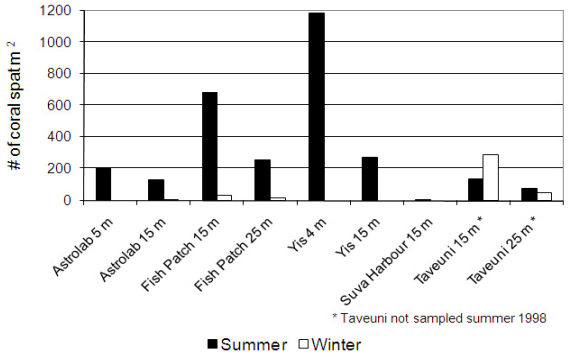

Figure 5: Number of Acroporidae recruits m^{-2} by site and season

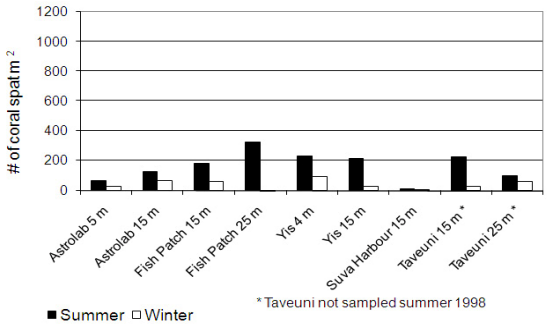

Figure 6: Number of Pocilloporidae recruits m^{-2} by site and season

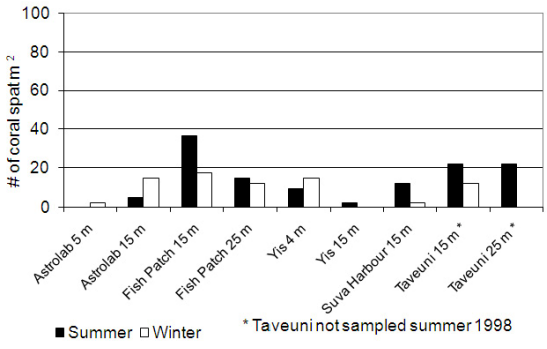

Figure 7: Number of recruits m^{-2} of Poritidae by site and season

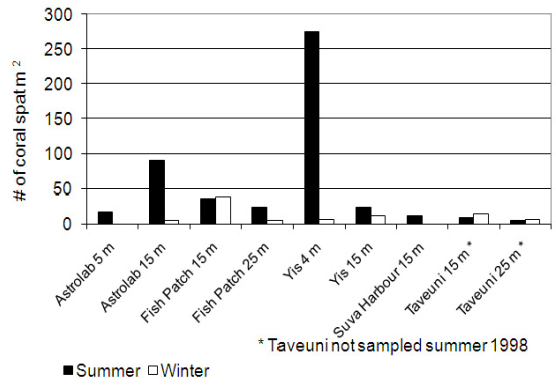

Figure 8: Number of "other" recruits m^{-2} by site and season

Discussion

The density of coral recruits at most sites in Fiji was higher than reported from other tropical sites using a similar sampling strategy (Kojis 1997, Quinn & Kojis 2002, 2003, 2008). The mean recruitment rate for summer (pooled across sites for summer 1997 and 1998) was $570m^{-2}$ (SD $\pm$ 519; N=9) compared with $31m^{-2}$ observed in Saipan; $0m^{-2}$, Rota; and $35m^{-2}$ Tinian in the Commonwealth of the Northern Mariana Islands (CNMI) in 1995 (Quinn & Kojis 2002) and $89\text{-}160m^{-2}$ in the eastern Caribbean (Kojis 1997). Even the mean winter recruitment ($93m^{-2}$) was over three times the "summer" recruitment recorded in the CNMI. The only Fijian site with a low recruitment rate was the turbid water site in Suva Channel. Davis et al. (1998) reported finding dangerously high levels of TBT in mollusks collected in Suva Harbour and other workers have reported on the pollution in the harbor (Naidu & Morrison 1994).

Several factors probably contribute to the high recruitment rate at most sites. Firstly, the sites were on reefs with high coral cover and a rich diversity of species. Secondly, there was an abundance of healthy, fecund reefs in close proximity releasing large amounts of larvae. The number of broadcast spawning acroporids recruiting was high and was probably related to the high frequency of occurrence of the family and the large numbers of larvae they produce.

The relative abundance of the various taxa of the recruits was similar to that reported from the GBR. Acroporid recruits were dominant in Fiji (52%) and in the Cairns region (16^{o} latitude) of the GBR (65%) followed by pocilloporids: 30% - Fiji and 25% - GBR, Cairns region (Fisk & Harriott 1990). The third most abundant group was the "other". More work is required to identify young spat of many families. Poritidae spat had a higher relative abundance (3%), then on the GBR at similar latitudes, (0%) (Fisk & Harriott 1990), but were less abundant (16%) than on an isolated sub tropical reef (Lord Howe Is., 31^{o}S) (Harriott 1992). We suspect this relates to differences in the relative abundance of adults of the family on the reefs at each location.

Coral recruitment was greater in the summer than in winter and this was consistent with the pattern for the GBR (Fisk & Harriott 1990). We hypothesize that most recruitment occurred during the main coral spawning season in October/November (Fiebig & Vuki 1998). However, brooding corals often have an extended spawning season (Harrison and Wallace 1990) and this is true of pocilloporid corals (Stoddart & Black 1985), which recruited in both winter and summer.

The high number of recruits on the Taveuni tiles, which were collected in November, was probably the result of the "winter" tiles still being deployed at the start of the summer spawning season for broadcast spawning species.

The reproductive life history of milleporan corals is poorly known. It is unclear whether the sporadic milleporan recruitment that was observed was due to random settlement of relatively few larvae or the unsuitability of the settlement surface for milleporan planulae.

The GAR sites represented communities that have developed within a very limited range of environmen-tal conditions. In addition to a narrow range of temp-erature, salinity, and dissolved oxygen, they are subjected to low sedimentation rates and concomitant low nutrient levels (Charpy et al. 1996). Chevillon et al. (1996) found no lithoclasts in the sediment of the GAR and concluded that there was limited terri-genous influence in the lagoon. However, none of their sample sites were within 1km of YIS, the site with the highest sedimentation rate. Sediment from soil erosion from the island is clearly evident at YIS.

While sedimentation has been shown to reduce fecundity in corals at depth (Kojis & Quinn 1984), the high sedimentation rate at YIS did not result in low recruitment rates. Although coral larvae are trans-ported between reefs (Fisk & Harriott 1990) as well as retained on the reef by water flow patterns (Black et al. 1991), it is unexpected that such high recruit-ment would occur on the site with the highest sedimentation rate. We suspect it is likely that this is because most of the sedimentation occurs during relatively short periods of high rainfall and, the effects probably dissipate rapidly, and do not affect reproduction. Also, there was no evidence of increased coral mortality or bleaching at YIS. The conservative mixing of reef waters within the lagoon, coupled with the synchronized mass spawning of abundant acroporids, may allow for greater fertile-zation success and retention of planulae resulting in higher recruitment rates. However, this does not explain the lower recruitment rates at the other lagoonal site near Dravuni within GAR. Given that the residence time for water mass in the GAR was 15 - 25 days (MacLeod

1992) and the sedimentation rate was lower at the other lagoonal site, one might reasonably expect a more uniform availability of larvae throughout the lagoon from at least broadcast spawning species and a more uniform rate of recruitment at both lagoonal sites.

Corals are known be have adapted to various levels of short-term exposure to sedimentation (Rogers 1983). Increased long-term sedimentation is generally associated with increased erosion associated with land modification or dredging and /or an increase in resuspended sediment. Chronic stress from sedimentation will result in lower coral fecundity (Kojis & Quinn 1984), lowering the number of larvae available for recruitment and reducing a coral reef community's ability to recover from natural perturbations. Additionally, the decreased water transparency associated with higher turbidity is known to reduce the depth range of species (Kojis & Quinn 1984) and cause bleaching and the death of corals (Rogers 1979). In the case of YIS, a reef receiving a high sediment load, the summer recruitment rate was about 3.5 times greater than at the two other GAR sites. Summer recruitment rates at the other two GAR sites were within 10% of each other.

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Experimental characterization of the water exchanges with ocean in a macro-tidal intermittently open lagoon bounded by semi-submerged coral reef

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Abstract. In the macro-tidal lagoons, of the Indian Ocean, coral reefs can be momentarily submerged by water at high tide and partially emerged at low tide. This process contributes to lagoon and open sea exchanges, although the reefs are often considered as impervious and the water fluxes assumed to occur only through the passes. To gain insight in spatial variability of fluxes at a reasonable cost that moorings alone cannot be able to provide, we develop an original experimental approach combining small ship side mounted ADCP measurement following transects thru passes and near reefs, with more classical high resolution ADCP moorings. This new strategy of measurement is exemplified at the occasion of an experimental campaign on the Mayotte lagoon and used after in Tulear lagoon. The results of this experiment are presented. Particularly, first, it is shown how the mounted ADCP data are made. A specific tidal analysis methodology is then proposed to get the spatial variability of the tidal component of the current thru the passes and above the reef. Then, all these analysis allow us to estimate the tidal induced fluxes thru the passes and above the reefs and to evaluate their respective part in water lagoon renewal.

Key words: measurement, tidal current, side mounted ADCP

Introduction

Tropical lagoons are impacted by climatic changes but also by direct anthropogenic activities. Water quality is directly related to water renewal time, which is regulated by passes and by the coral reef barrier. Possible reef modification (anthropic or intense climatic phenomena induced destruction, sudden stop of the suited general conditions for their edification, water level increase) could have major consequences on the functioning of reef-lagoon. To be able to forecast the reef tolerance versus modifications of the lagoon functioning conditions, it is needed to understand the hydrodynamic regulation induced by these reef barriers and the possible evolution of their regulating effects.

At some meso or macro tidal lagoons of the Indian Ocean, the water that is over running above the reef strongly tidally modulates fluxes through passes. The Mayotte lagoon and the Tulear lagoon, situated in the Mozambique Channel, are examples of meso-tidal reef lagoons. The pronounced tidal character of these lagoons generates peculiar hydrodynamics, which depend upon the tidal cycle.

In this study, it has been decided to focus on the cross reef tidal fluxes on the Mayotte lagoon and the Tulear lagoon. Our aim is to know the influence of the reef hydrodynamic control on the open seawater exchanges and on the lagoon dynamics, as well as to compare flux above coral reef and through passes.

In order to gain insight into spatial and temporal variability of fluxes at a reasonable cost that moorings alone are not able to provide, we have developed an original experimental approach. This approach combines small shipside mounted ADCP measurement following transects with more classical high-resolution ADCP moorings. This experimental strategy will be first presented, and then the specific analysis methodology will be described. In the third part, results will be presented and discussed, mainly, the tidal induced fluxes through passes and above reefs, as well as their respective part in water lagoon renewal**.**

Field experiment

Study site

The field observations were performed at the Tulear lagoon and at the north-east lagoon of Mayotte located in the Mozambique Channel. The north-east lagoon of Mayotte constitutes the north-eastern part of a wider lagoon extended to the south. These two

lagoons that are located in same broad geographical area, are submitted to a quite similar external tide, dominated by the semi-diurnal wave M2.

Even if their orientation is opposite, their morphology is similar. They are about a 10nm long and 1nm to 5nm wide, opened at two passes of about 1 to 2nm. The north pass of the Mayotte lagoon and the south pass of the Tulear lagoon are the less wide (1nm) and the deeper ones (50m at the Mayotte lagoon; 20m at the Tulear lagoon). These lagoons are partially closed to the Mozambique Channel along the long reef. The Mayotte reef is a well-developed fringing reef whereas the Tulear reef is mainly a sand and mud covered, dead reef. Its topography is broken with crest and moat. Even if the geological origins of this reef, the external aspect or the width are different, their influence in the flow control seems to be the same. At high water, the reef is submerged (2-3m at the Mayotte lagoon), whereas the reef is weakly immerged (50 cm at the Mayotte lagoon) or emerged (at the Tulear lagoon) at low water.

However, the Mayotte lagoon is deeper (about 20m) than the Tulear one (about 10m), and the nature of entrance water varies with lagoon. At the Tulear lagoon, the incoming flow is usually constituted by water from the Mozambique Channel, but sometimes, due to external condition such as wind, a weak quantity of fresh water from Fiherena and Onilahy Rivers could enter. At the Mayotte lagoon, the incoming water could be either Mozambique Cannel water (entering through the north pass and above the reef) or lagoon water from the south lagoon (entering through the south pass).

Sensor deployment

Our observations were performed from 2006, November 9th, to 2006, November 24th, at the Mayotte lagoon and from 2007, September 9th to October 12th at the Tulear lagoon. To obtain spatial and temporal variability of hydrodynamics, we deployed moorings on which ADCP were installed and associated hull-mounted ADCP measurements. To investigate the flow through the pass and above the reef, we conducted a field survey along pass and along the reef.

Moorings deployment:

At the Mayotte lagoon, the north pass is divided in two channels. One ADCP was moored in each channel. The third was moored in the south pass and the last one, along the reef. At the Tulear lagoon, the south pass is divided in two channels as well. One ADCP was moored in each of them, the third one in the north pass. Because of technical problem, the last one was not moored along the reef, but in the middle of the lagoon. Finally, in each lagoon and during each campaign, four ADCP were deployed at locations indicated in Fig 1. In order to compare the velocity measured with moored ADCP and with side mounted ADCP, the cell had been set to 1m. About ten cells were obtained for each ADCP.

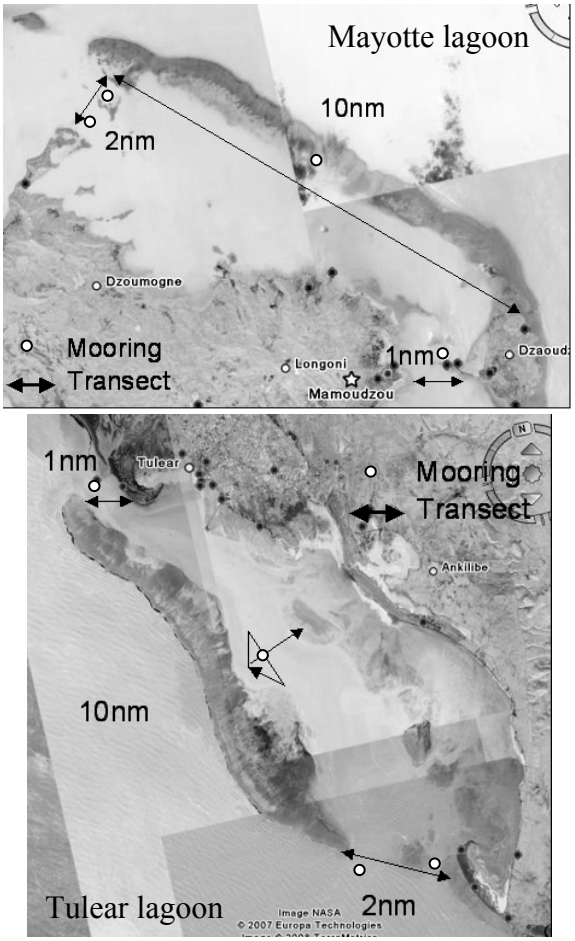

Figure 1: Study site (Mayotte lagoon and Tulear lagoon) and sensor deployment

In order to lighten the ADCP deployment field, the ADCP was fixed into a red plastic box weighted with concrete bloc. With only two divers and a small boat as a zodiac, this mooring can be deployed. Even with this rudimentary aspect and with velocity able to reach 2m/s, these moorings had been stable and a mooring set for tests did not move for more than 6 months.

Description of transect:

As is usually done to measure currents in rivers, the ADCP was set up on a boat with a tubular stainless steel frame along the inflatable fender. In low waves and with a boat velocity of about 3 knots, measurements were in good agreement with measured velocity mooring. Then, to associated data from the side mounted ADCP with those from mooring and to

quantify fluxes, transects were made near moorings, along passes and along the reef barrier (at the Mayotte lagoon) or in the middle of the lagoon (at the Tulear lagoon) as presented in Fig 1.

Data description

The tide is mainly semidiurnal with a fluctuation spring and neap tide. The campaign duration was chosen to measure this variability. In Mayotte, the tidal amplitude is about 2m during the spring tide and 0.5m during the neap tide. In Tulear, the tidal amplitude is weaker, about 1.50m during the spring tide and 0.25m during the neap tide.

Velocity at moorings was obtained in the two lagoons. The Mayotte campaign was quite short in time and measurements were only performed during 10 days. But, the Tulear campaign was much more optimized and moored measurements were far longer (until 50 days). During the November Mayotte campaign, at the end of the rainy season, the sea was quite calm, while, during the October Tulear campaign, the sea was rougher, but transects could be performed. Then, velocity-measurements along transect were successful, but due to technical problems some data had not been acquired.

Velocities are quasi-barotropic; their main direction is cross-pass or cross-reef at the boundaries and along-shore in the middle of the lagoon. Velocities are correlated to the tide. Periodograms can reveal the mean periodic waves. The main water level tidal waves are M2, S2 and K2 in Mayotte and mainly M2 and K2 in Tulear. The velocities are modulated by these waves, but other harmonics of M2 or S2 can be observed (notably, waves with a period of 6h).

The side mounted ADCP gives spatial information about the velocity along the pass. These measurements reveal variability due to the topography of the bottom.

Data processing.

The side mounted ADCP gives a spatial information velocity, however not synoptically. Indeed, the transect duration can take 1 hour. Notably, due to the tide effect, the velocity varies during this hour. Then, it is hard to compare velocity measured at the beginning of transect with the one measured at the end without treatment. So to treat this spatial information, the velocities have to be estimated for the same time for each transect. For this purpose, the temporal information of the mooring can be used to obtain temporal information at each point all along the transect.

Spectral analysis of mooring data

At the mooring station, named M_0, the measured velocity, is not the real velocity, but an approximation: $\hat{u}(M_0) = u(M_0) + \varepsilon(M_0)$, with ε, the measurement error with a blank noise and $u(M_0)$, the complex representation of the velocity: $u(M_0) = \|\vec{u}(M_0)\| e^{i\theta(M_0)}$. Supposing a linear decomposition, the velocity can be decomposed into a periodic signal, u_{tide}, mainly linked to the tide and a non-periodic signal, $u_{res}(M_0)$:

$$u(M_0) = u_{tide}(M_0) + u_{res}(M_0).$$

The main frequencies of velocity waves can be deduced from the periodogram. Then, a classical harmonic analysis can allow reconstructing the tidal signal of this velocity.

$$u_{tide}(M_0) = \sum_{k=1}^{N} A_{M_0}(\omega_k) e^{i\omega_k t + \varphi_k}$$

with ω_k, $\varphi_k(M_0)$ and $A_{M_0}(\omega_k)$ the pulsation, the phase and the amplitudes for wave k and N the number of waves which depends on the tidal dynamics of area studied.

Wave amplitudes can be approximated by $A^*_{M_0}(\omega_k)$ with minimizing, by the least square sense, the equation:

$$u(M_0) - \sum_{k=1}^{N} A_{M_0}(\omega_k) e^{i\omega_k t + \varphi_k}$$

Hence, the approximated tidal velocity at M_0 location, $u^*_{tide}(M_0)$, and the approximated "residual velocity", $u^*_{res}(M_0)$, are written:

$$u^*_{tide}(M_0) = \sum_{k=1}^{N} A_{M_0}(\omega_k) e^{i\omega_k t + \varphi_k}$$

$$\text{and } u^*_{res}(M_0) = u(M_0) - u^*_{tide}(M_0)$$

$u^*_{res}(M_0)$ represents not only the measurement errors or harmonic analysis approximation, but also all velocity component not represented with the harmonic analysis as velocity due to wind effect or non-represented tidal wave, for example. Hence, the approximated velocity at M_0 is:

$$u^*(M_0) = u^*_{tide}(M_0) + u^*_{res}(M_0)$$

Temporal and spatial data processing

As identical transect were repeated along the campaign, many instantaneous data can be obtained in one location. The method proposed here is an expanded version of Candela et al. 1992 and Garcia-Gorriz et al., 2003. The main difference being that their method was developed in meso-scale region and not in coastal region and contrary to the both technical; our strategy uses a reference mooring. As for the velocity at the mooring, velocity at each point of the transect, named M, is assumed to be expanded

in two types of components: time periodic and non-periodic component. Both are spatially variable. Then, equations analogous to the equation for the velocity at mooring, can be written at each transect location, M. Hence:

$$\hat{u}(M) = u(M) + \varepsilon(M),$$

with $\hat{u}(M)$ the measured velocity in M location, $\varepsilon(M)$ the measurement error estimated to about 5cm/s and $u(M)$ the real velocity. Then, the velocity is decomposed as tide-induced velocity, $u_{tide}(M)$, and "residual velocity", $u_{res}(M)$:

$$u(M) = u_{tide}(M) + u_{res}(M).$$

The aim is to estimate the tide-induced velocity and the "residual velocity". For that, as Candela et al, 1992 and Garcia-Gorriz et al, 2003, we assume that the time-periodic part of the equation can be expressed as the product of amplitude, and a sinusoidal function of time.

$$u_{tide}(M) = \sum_{k=1}^{N} A_M(\omega_k) e^{i\omega_k t + \varphi_k(M)},$$

with $\varphi_k(M)$ and $A_M(\omega_k)$ the phase and the amplitudes for the wave *k* at the location M. N is the number of waves which depends on the tidal dynamics of area studied and which is the same as for the velocity at the mooring.

$A_M(\omega_k)$ and $u_{res}(M)$ are functions depending on the location of M. Here, *s,* the distance from the mooring, characterizes the location: Candela et al, 1990 show that the quality of the fit was not strongly dependent on the choice of the function. Thus, the horizontal functions $A_M(\omega_k)$ and $u_{res}(M)$ are prescribed and we chose them to be polynomials. They are based in both velocity at the mooring location and along transect with no other dynamical assumption involved, and are expanded as

$$A_M(\omega_k) = A^*_{M_0}(\omega_k).P_k^{(n)}(s)$$

$$\text{and } u_{res}(M) = u^*_{res}(M_0).Q^{(m)}(s)$$

with $P_k^{(n)}(s)$ and $Q^{(m)}(s)$ polynomial expressed as:

$$P_k^{(n)}(s) = \sum_{p=1}^{n} \alpha_{p,k} s^p$$

$$\text{and } Q^{(m)}(s) = \sum_{p=1}^{m} \beta_p s^p,$$

where $\alpha_{p,k}$ and β_p are the constants to be fitted to the observations and n and m the degree of the polynomials.

Setting,

$$J(\alpha_{p,k}, \beta_p) = \left\| \sum_{k=1}^{N} A^*_{M_0}(\omega_k).\left(\sum_{p=1}^{n} \alpha_{p,k} s^p \right) e^{i\omega_k t + \varphi_k(M)} + u^*_{res}(M_0).\sum_{p=1}^{m} \beta_p s^p - \hat{u}(M, t) \right\|^2$$

$\alpha^*_{p,k}$ and β^*_p are defined as the values minimizing J.

Then, the approximated tidal and residual velocity at M location, ($u^*_{tide}(M)$ and $u^*_{res}(M)$), are written:

$$u^*_{tide}(M) = \sum_{k=1}^{N} A^*_{M_0}(\omega_k).\left(\sum_{p=1}^{n} \alpha^*_{p,k} s^p \right) e^{i\omega_k t + \varphi_k(M)}$$

$$\text{and } u^*_{res}(M) = u^*_{res}(M_0).\sum_{p=1}^{m} \beta^*_p s^p$$

Hence, the approximated velocity at M is:

$$u^*(M) = u^*_{tide}(M) + u^*_{res}(M)$$

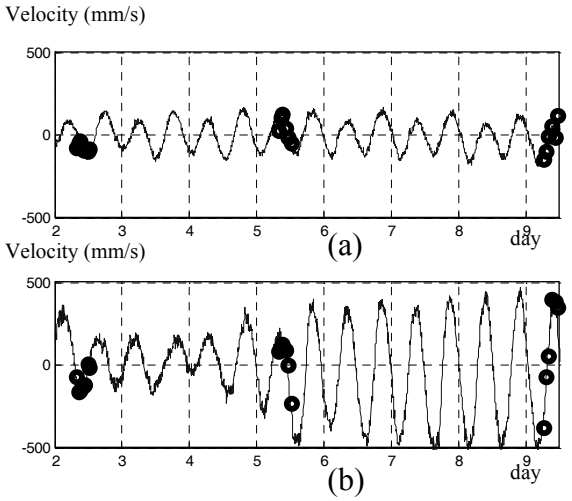

Figure 2: Measured velocity (black points) and calculated velocity (black line) in the middle of the south pass of Tulear lagoon. (a) represents the east component and (b) the north component.

As Garcia-Gorriz and al (2003), a systematic criterion was used to select the degrees of the polynomial. This test computes the error and the correlation when increasing the degree of polynomial functions. Hence, sequential tests with increasing polynomial degree values are run and the choice is made at the step when correlation tends towards 1 and errors displays small variation for higher degrees. The degree of the polynomial functions in the equation depends on the circulation in the studied area. Moreover, if M_0 is located on transect, $\alpha^*_{0,k}$ and β^*_0 are set to 1. Fig. 2 illustrates the reconstituted velocity in one point with the mooring velocity and in-situ data.

Results and discussion

Velocity

The resulting velocities are in accordance with measurement. The correlations with data are quite good (around to 0.9) and the error is weak (between 3 and 4 cm/s). The resulting velocity provides the main features of the velocity in passes. In the Tulear lagoon, the velocity is almost homogenous and follows the bathymetry. Contrarily, at the south pass of the Mayotte lagoon, the velocity is much more complex and this data process can point out the eddy in front of the north pass (Gourbesville and Thomassin, 2000). Fig. 3 illustrates the velocity at the Mayotte lagoon and the Tulear lagoon.

Flux

Spatial and temporal informations of velocity allow calculating fluxes. During the tidal campaign, in Mayotte, a mean flux of about 3000m^3/s entranced from the north pass whereas a flux of 2000m^3/s above the reef and of 1500 m^3/s through pass went out from the lagoon. At the Tulear lagoon, the flux went out through pass with a quantity of 1000 m^3/s and 2000 m^3/s. Hence, the water renewal times were respectively 7 and 4 days.

Conclusion:

A new strategy of data acquisition and a new data processing development to obtain spatial and temporal variations of current was developed. This methodology can provide spatial and temporal hydrodynamics information at reasonable cost. This data processing associates mooring and side mounted ADCP and evaluates the through-pass velocity with polynomial functions whose coefficient depends on the velocity at the mooring. Then, results can be extrapolated without any transect and the presented measurements allow calculating entrance lagoon flux. Moreover this boundary description allows the building of a numerical model that will be used to compute the residence time of water masses.

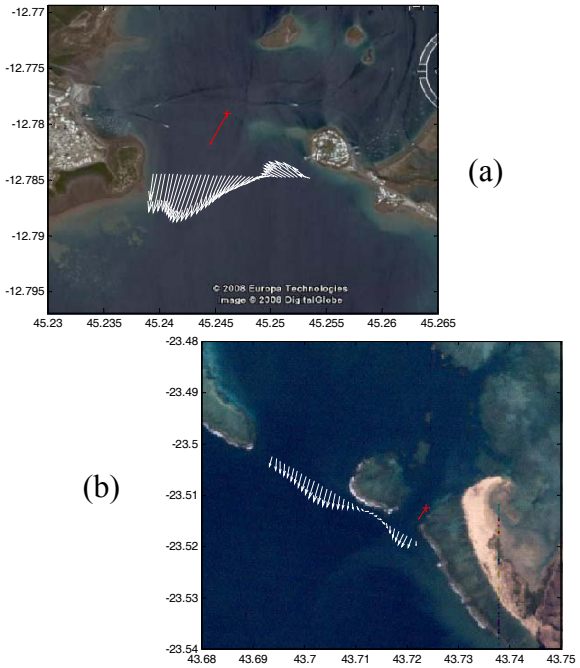

Figure 3: Reconstituted velocity in the south pass of Mayotte (a) and Tulear (b) lagoons.

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Characterization of biologically significant hydrodynamic anomalies on the Florida Reef Tract

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Abstract. The U. S. National Oceanic and Atmospheric Administration (NOAA) Integrated Coral Observing Network (ICON) Project uses artificial-intelligence software to implement heuristic models of coral reef ecosystem response to physical conditions. These models use if-then rules to recognize patterns in environmental data integrated in near real-time from multiple sources. One model is described to detect episodic, biologically significant fluxes acting upon coral reefs in the Florida Reef Tract. Data are gathered from *in situ* sensors and satellites for three sites near the reef crest: Sombrero Key in the Middle Keys, Molasses Reef in the Upper Keys, and Fowey Rocks off Miami. The model recognizes apparent circulation changes that may impact reef ecology. Criteria are *in situ* sea-temperature variability at near-tidal frequencies, wind velocity variability, and color-derived satellite chlorophyll *a* point data. Model ecological forecasts (*ecoforecasts*) are verified using secondary data not input to the model, including satellite ocean-color imagery, radar-derived ocean surface currents, and divers' reports. Events are characterized as being one of wind-driven upwelling; net transport of eutrophic water from outside the FRT; and interaction of Florida Current frontal features with reef topography, possibly modulated by internal wave-breaking. Multiple events are characterized in a 42-month period in 2005-2008.

Key words: coral reef • NOAA • chlorophyll • eddies and vortices • tidal mixing

Introduction

The Florida Reef Tract (FRT) encompasses the third largest barrier reef system in the world (FKNMS, 2001). Offshore, the Florida Current (FC) flows through the Straits of Florida after interacting with marine environments and ecosystems throughout the Caribbean Sea and the Gulf of Mexico. Meanders of the FC and mesoscale eddies interact with sloping topography in and offshore of the FRT (Lee et al. 2002). Smaller-scale vortices and coastal waves inshore of the FC front (Shay et al. 2007), and various upstream rivers (Hu et al. 2004, 2005) are also observed to interact with the reef as well. Finally, the Keys island chain is crosscut from the north by shallow channels, which episodically admit waters onto the back reef of the FRT from the West Florida Shelf (WFS) and outer Florida Bay (Smith and Pitts 2002; Lee et al. 2006).

The dynamics of circulation between the WFS, the FRT, and the terraces and deeper waters of the Straits can be highly complex, as are the biological responses of the reef ecosystem to the resulting fluxes of allochthonous water. These complex interactions can impact water quality as well as the marine ecology of the Keys, with important implications for coastal resource management within the region. Improvements in the characterization of these interactions are critical to the scientific understanding of nutrient dynamics and larval recruitment on the FRT. These processes, in turn, have important economic and ecological consequences, with potentially significant impact on tourism and commercial fishing interests.

An important tool for understanding this complex system is the Sustained Ecological Research Related to Management of the Florida Keys Seascape (SEAKEYS; Ogden et al. 1994) network of automated monitoring stations. These stations are installed upon navigational structures along the length of the FRT, providing a permanent infrastructure for continuous monitoring of meteorological and hydrographic environmental conditions. Three of these SEAKEYS stations, Sombrero Key, Molasses Reef, and Fowey Rocks, are located immediately inshore of the outer reef crest in the Middle and Upper Keys, providing a valuable base from which to

observe physical interactions between the FC front just offshore, and the shallow basin of the back reef just inshore. Furthermore, two of these stations, at Sombrero Key and Molasses Reef, are also suitably positioned to monitor hydrographic and ecological effects due to fluxes from the WFS and outer Florida Bay through the bridge channels of the Florida Keys.

Data

All SEAKEYS stations transmit data hourly in standard C-MAN format, via NOAA geosynchronous operational satellites. These transmissions are archived by the NOAA National Data Buoy Center (NDBC), and are analyzed in near real-time at NOAA's Atlantic Oceanographic and Meteorological Laboratory (AOML). The data reported by each SEAKEYS station includes hourly mean wind speed and direction, depth-averaged *in situ* sea temperature, and in some cases, *in situ* salinity, tidal height, or sea-surface Photosynthetically Active Radiation (PAR). The archived SEAKEYS data provide a nearly continuous record of these environmental variables, stretching in some cases as far back as 1987 (NDBC 2008).

The ICON Project has worked with collaborators at University of South Florida's College of Marine Science, Institute for Marine Remote Sensing (USF 2008) to integrate remote sensing data from the satellite-borne MODIS and AVHRR instruments. These products are geo-located and subsetted to each individual monitoring station location, to allow direct integration in time and space with *in situ* data. They provide high spatial resolution (order of 1km) data on sea surface temperature (SST) and ocean-color derived chlorophyll *a* concentrations, downloaded to NOAA AOML within 12 to 24 hours of measurement and indexed to individual reef sites.

Methodology

These integrated data streams are analyzed by AOML. They are evaluated using expert-systems software and heuristic if-then rules developed at AOML (Hendee et al. 2001, 2008) for both quality control, and for the presence of environmental conditions that may have an impact on the coral reef ecosystem, on the marine environment, and on important maritime economic activities in the region. The outputs of these if-then models are ecological forecasts (called *ecoforecasts*). This suite of data-integration, quality control, and near real-time monitoring techniques is the basis for the present study.

Statistical processing is then applied to the raw environmental variables, to derive time series of indices that can be used to directly assess certain hydrodynamic and biological conditions at the monitoring site. The primary index used to indicate increases in biological productivity on the reef over periods of one to three days is chlorophyll *a* concentration (chl_a). The USF-CMS chl_a product provides numerical estimates in $mg \cdot m^{-3}$, based on near real-time analysis of Terra and Aqua MODIS ocean color measurements. In this complex environment there is large uncertainty in the absolute value of chl_a, but here it is used as a relative index to show spatial and temporal patterns. High spatial-resolution time series of satellite chl_a for each of the three SEAKEYS sites have been derived for the sample period of 2005-2008. These time series are then filtered using quality control procedures similar to those used by the ICON Project for other types of *in situ* data. Peaks of this quality-controlled chl_a that fall above the 80^{th} percentile for the entire record at each site are then flagged as indicators of change in biological productivity at that site.

For each hourly average sea temperature value in the SEAKEYS *in situ* record, a simple subsample of the values from the prior 24 hours is collected, and the standard deviation of this sample is calculated. A 3-day simple moving average is applied to smooth this *sea temperature variability* time series. This procedure is intended to filter for episodes of sustained variance in sea temperature near inertial and dominant tidal frequencies. Such events include sustained anomalies, where either unusually cold water (Fig. 1) or unusually warm *and* cold waters (Fig. 2) are circulated onto the reef. One possible cause for near-tidal period, high-variance, *cold* events is the breaking of baroclinic tides (internal waves) on the reef slope (Leichter et al. 1996, 2003). When a meander or vortex associated with the FC has previously transported cooler waters to the base of the reef slope, such internal waves can transport and mix those waters up the reef slope. A likely cause of horizontal-mixing (warm *and* cold-anomaly) events is action of the barotropic tide interacting with shallow reef-crest topography. Periods of high chl_a (see above) that do not also correspond to periods of high sea temperature variability are flagged by the expert system as being of particular interest, as these may indicate instances where nutrients of more localized origin are causing a spike in productivity.

Wind velocity is also analyzed to distinguish events of anomalously high chlorophyll *a* concentration and/or high sea temperature variability that can be attributed to wind-forcing, as opposed to other proximate causes such as tidal mixing and frontal interaction with reef topography. Individual zonal and meridional wind vector components are derived from each hourly *in situ* wind-speed and direction measurement. Three-day subsamples of these U_{10} and V_{10} component values are then collected for each hour of the record, and a standard deviation of those

subsamples is calculated. The result is intended to provide an hourly index for *impulses* of substantial wind stress forcing that are of sufficient magnitude and duration to produce anomalously high wind-driven (shallow-water) currents in the back-reef, and/or wind-driven upwelling events on the fore-reef slope and reef-crest.

Results

Ecoforecasts were produced by the heuristic model independently for each of the three monitoring sites, for each day between 2005 Jan 01 and 2008 Jun 30 when statistically significant high sea temperature variability was indicated. Output for each ecoforecast included three components: 1) all antecedent data values (chl_a in $mg \cdot m^{-3}$, sea temperature variability index, wind impulse index); 2) a numerical indicator of the relative intensity of the forecast ecological response, called a Stimulus/Response Index (S/RI; Hendee et al. submitted); and 3) a series of symbolic codes indicating the attempted classification of each ecoforecast according to *dynamical mechanism* (wind-forced or non-wind forced), and *productivity* (sea-temperature variability coincident with high chlorophyll, or not).

Second, where a significant ecoforecast was produced for multiple days in a row at a given site, ecoforecasts were grouped together into an extended *event*. An attempt was then made to verify the dynamical mechanisms forecast for each event using synoptic satellite imagery of the Straits of Florida (Fig. 3), and where available, 1km-resolution ocean surface current vector fields derived from WERA High-Frequency radar (Shay et al. 2007). These images and fields were then further examined to characterize the regional scale of such events and any translational motions apparent in the associated phenomena, such as visible vortices offshore of the reef crest, or extended plumes of high chlorophyll *a* and turbid water inshore in the back-reef.

At Sombrero Key Reef, 44 such events were confirmed in the sample period, comprising 273 individual days of high sea temperature variability. At Molasses Reef only 28 events totaling 108 days were observed. Finally, at Fowey Rocks, the site furthest downstream relative to the FC, 38 events comprising 133 days of high sea temperature variance occurred.

The dynamical mechanism indicated by the ecoforecasts also showed significant variability between sites, with wind velocity variability strongly indicated as a forcing mechanism for 32% of all events at Molasses Reef, but only 21% of events at both Sombrero Key and Fowey Rocks. Overall, sea temperature variability events were at least partially coincident with high chlorophyll *a* concentrations 80% of the time across the three sites. Finally, although it was beyond the scope of the present study, periods of high chl_a concentration were also noted where there was *no* significant sea temperature variability. These also showed variability of incidence between sites – but with Sombrero Key showing significantly lower correlation between sea temperature variability and chl_a than the other two sites did.

Discussion and Conclusion

The pattern of spatial variability in the results is suggestive of possible differences in the dynamical regimes operating at each reef site. Sombrero Key is a site that is believed to be subject to periodic, persistent and significant influence from WFS, due to its proximity to the relatively large Moser Channel beneath Seven Mile Bridge. It is also a site where mesoscale vortices, long-period surface wave activity, and dominant wind forcing during much of the year may all be favorable to upwelling and up-mixing of nutrients from deeper FC waters offshore.

Molasses Reef by contrast is a site where the angle of the reef-crest isobaths to wind stress forcing differs by approximately 45^{o} from that at Sombrero (Lee et al. 2002). This cyclonic bend in topography, together with horizontal broadening in offshore isobaths, may also explain why this and other Upper Florida Keys sites are subject to relatively less mesoscale and sub-mesoscale cyclonic vorticity than Lower Keys sites (Fiechter et al. 2008). This is significant as such vorticity is also a potential forcing mechanism for upwelling of cool water and nutrients when it interacts with steep reef slope bottom topography.

The angle between reef crest isobaths at Fowey Rocks and the wind vector is shifted still further, by approximately 90^{o} relative to Sombrero Key. Compared to the isobaths at Molasses, however, bottom topography offshore of Fowey is also relatively steep, more like that at Sombrero. These facts are represented in our results, both by the intermediate frequency of events, and by the relatively low incidence of those events where wind was implicated as a dominant forcing mechanism.

Overall, the heuristic modeling approach is a powerful mechanism for monitoring and interpretation of both historical and near real-time environmental data on coral reefs. Furthermore, the indices chosen for the ecoforecast model described in this text, while kinematical and categorical, still appear to provide some valuable insights into the dynamical regimes operating on and affecting those reefs. Future work using these tools, coupled with additional data such as radar-derived surface currents, ship data, and numerical models of the mesoscale and sub-mesoscale circulation patterns on the reef, should bear still richer fruits.

Finally, these ecoforecasting tools are seen to have the potential to serve up useful information on marine conditions and reef ecology – to researchers, natural resource managers, and the public. Such information, made available in near real-time via the Web (ICON, 2008), can represent a unique perspective on the fragile ecosystem of the reef, and human use of it.

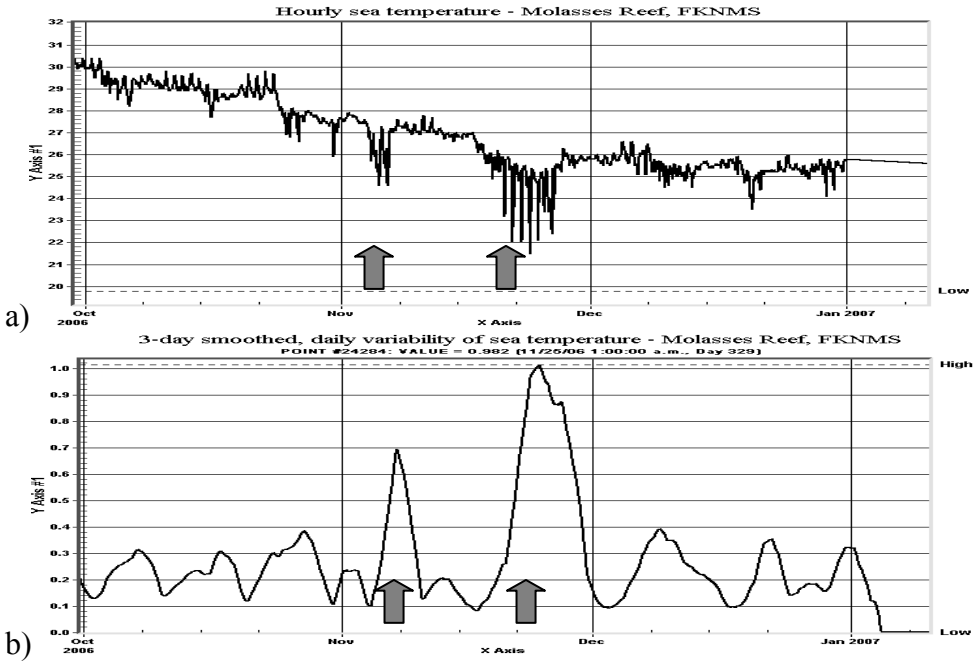

Figure 1: Cold-water anomaly (panel a) and associated peak in sea temperature variability index (b) associated with a likely upwelling event. Note that use of a realizable time-series filter in the variability index means it may lag raw sea temperature changes by one or more days.

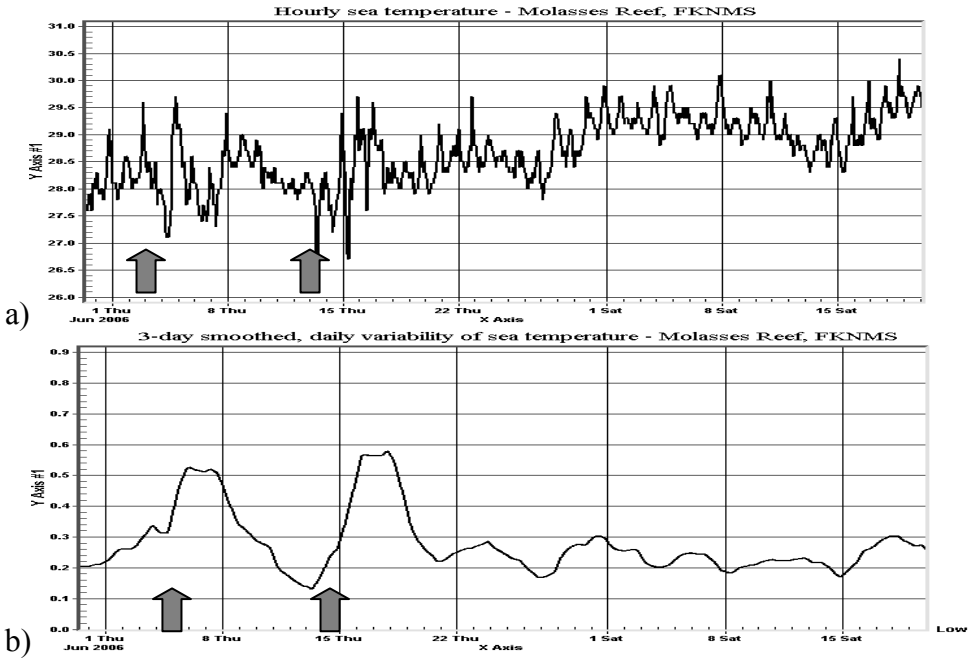

Figure 2: Sea temperature (a) and sea temperature variability index (b), showing a pair of events likely due to horizontal mixing of warmer (back-reef) and cooler (offshore) waters across the reef crest.

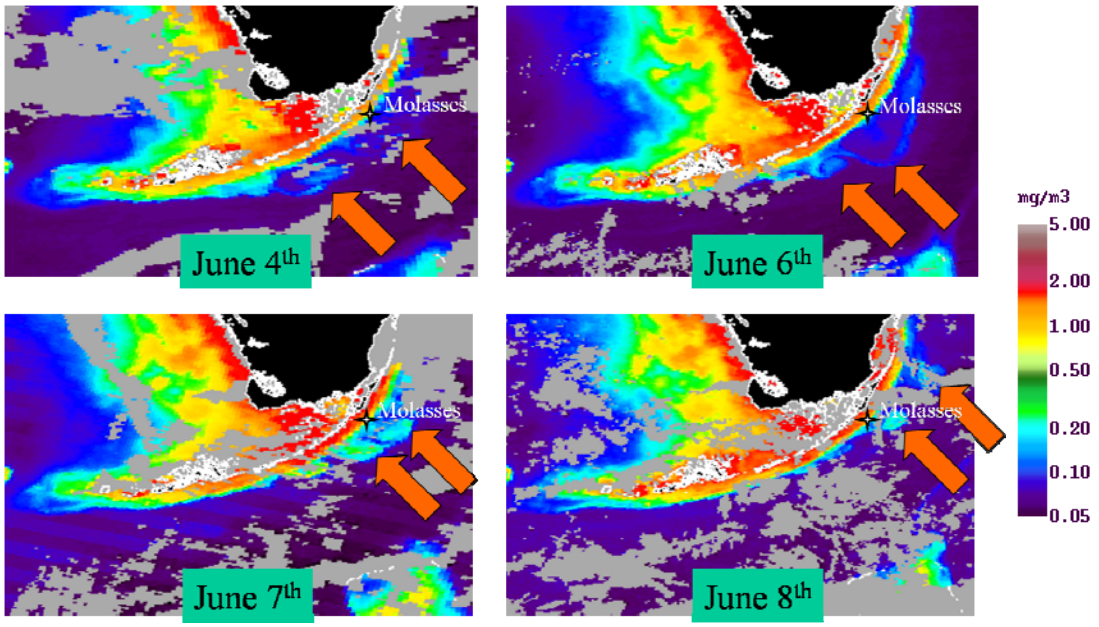

Figure 3: Sequence of MODIS satellite ocean-color images, showing chlorophyll *a* concentrations and associated circulation patterns in the Straits of Florida from 2006. Grey regions indicate cloud cover. Note the pair of vortices (arrows) translating past Molasses Reef over the four-day period, and interacting with one another and the inshore topography as they pass. These were among approximately 800 images examined visually in response to sea temperature variability ecoforecasts produced by the system described in the text, and served as visual confirmation both for the events, and the likely forcing mechanisms associated with each.

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Influence of Florida Current frontal eddies on circulation and fish recruitment around the Florida Keys Reef Tract

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Abstract. The coastal seas around the Florida Keys Reef Tract exhibit complex dynamics resulting from the interaction with the Loop Current/Florida Current system and the frontal eddies that interact with the complex reef topography. Nesting to a succession of coarser, regional model and finally to basin-wide and global models allows the downscaling of large scale flows to scales of reef related processes. A high resolution (~900m) of the Hybrid Coordinate Ocean Model has been developed for the Florida Keys (FKEYS-HYCOM).

Florida Current frontal eddies are important mechanisms for the interaction of nearshore and offshore flows. They enable upwelling in the vicinity of the Reef Tract and influence transport and recruitment pathways, as they carry waters of different properties (such as river-borne low salinity/nutrient-rich waters) and waters containing larvae from upstream source, or entrained from nearby spawning grounds. FKEYS-HYCOM simulates both mesoscale and submesoscale eddy passages during a 2-year simulation period (2004-2005), forced with high resolution/high frequency atmospheric forcing. Coupling with the ecological population connectivity BOLTS model (BiOphysical Lagrangian Tracking System) allows simulations of larval transport, taking into account not only the dispersion of active physical larvae, but also the interaction of factors influencing larval survival, habitat selection and condition at settlement.

Key words: Florida Current frontal eddies, larval transport, Reef Tract, FKEYS-HYCOM, upwelling

Introduction

The ocean circulation around South Florida is dominated by the Florida Current, which originates where the Loop Current enters the Straits of Florida from the Gulf of Mexico, subsequently becoming the Gulf Stream as it emerges in the South Atlantic Bight (Fig. 1). The Florida Bay is openly connected to the Southwest Florida Shelf along its wide western boundary, but exchange with the Atlantic coastal zone of the Keys is restricted to a few narrow passages between the Keys island chain. Freshwater runoff occurs along the Ten Thousand Islands area through a series of small rivers (Fig. 1). Loop Current eddies propagate southward along the outer edge of the Southwest Florida Shelf and develop into persistent eddy structures off the Dry Tortugas.

The Keys coastal zone consists of a narrow, curving shelf with complex topography associated with its shallow Reef Tract. The outer shelf region of the Keys is dominated by meanders of the Florida Current and the downstream propagation of eddies.

The Florida Current frequently forms cyclonic, cold-core frontal eddies along the Slope and Shelf. These eddies are formed along the western edge of the Florida Current and travel northward along the continental margin.

Recirculation within mesoscale eddies and their several months' duration in the Keys coastal zone have been proposed as a retention mechanism that may contribute to local recruitment of larvae spawned in the Dry Tortugas or along the outer Reef Tract (Lee et al. 1992). Off the Florida Keys, frontal eddies are associated with cross-shelf larval transports (Sponaugle et al. 2005).

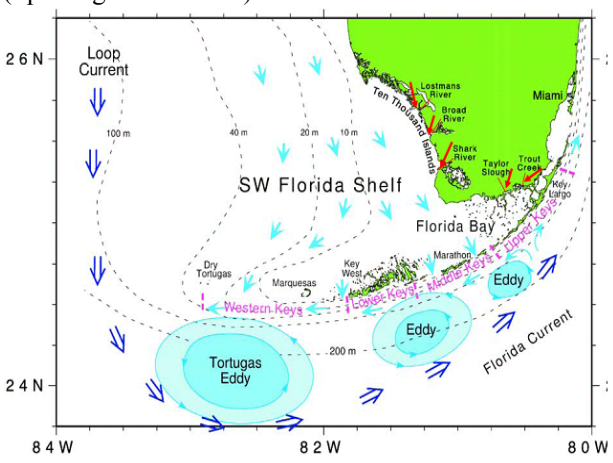

Figure 1. The complex South Florida coastal system, adapted from Lee et.al (2002). Red arrows mark the river sources.

A nested modeling approach has been employed to ensure the proper representation of the complex south Florida coastal system (Fig. 1). A high resolution (~900m) application of the Hybrid Coordinate Ocean

Model has been developed focusing on the circulation around the Florida Keys (FKEYS-HYCOM). Nesting to a succession of coarser, regional models (South Florida SoFLA-HYCOM and Gulf of Mexico GOM-HYCOM) and finally to basin-wide and global models allows the downscaling of large scale flows to scales appropriate for the study of reef related processes.

Model domain and set-up

The Florida Keys HYCOM model domain covers 79.0°W to 83.4°W and 22.8°N to 26.1°N (Fig. 2) with very high resolution of about 900m in horizontal and twenty-six hybrid-layers in vertical. It is being nested within the 1/25° SoFLA-HYCOM (Kourafalou et al. 2008) with daily updating for barotropic and baroclinic boundary conditions. Time integration steps are 30 sec for the baroclinic mode and 1 sec for the barotropic mode.

The topography is derived from the 2-minute NRL DBDB2 global dataset with a minimum depth of 2m. The FKEYS-HYCOM is forced with three-hourly wind stress, air temperature, atmospheric humidity, heat fluxes and precipitation from the 27-km horizontal resolution Coupled Ocean/Atmosphere Mesoscale Prediction System (COAMPS, Hodur 1997; Hodur et. al 2002) for the year 2004 and 2005.

River runoff from the Shark River in the domain is the important source of low salinity which can impact the water property inside/around Florida Bay and along the Florida Keys through the neighboring Keys passages. It was prescribed as a line source of fresh water input along the Ten Thousand Islands starting from the Shark River. Low salinity waters from the SW Florida Shelf reach the AFKS through the Keys passages, influencing water properties along the Reef Tract.

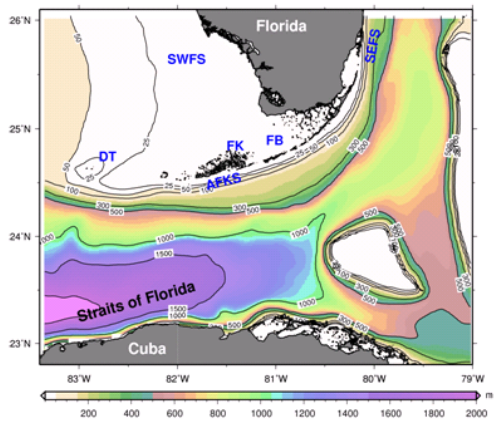

Figure 2. The Florida Keys (FKEYS) model domain and bathymetry (contours in m). It covers the Southwest Florida Shelf (SWFS), the Southeast Florida Shelf (SEFS), the Atlantic Florida Keys Shelf (AFKS) and the Strait of Florida, extending from Cuba to the Biscayne Bay. (FB: Florida Bay, FK: Florida Keys National Marine Sanctuary, DT: Dry Tortugas Ecological Reserve).

Evolution of eddies in the Straits of Florida

The Okubo-Weiss parameter is employed to quantify eddies and their propagation along the Florida Current front. This parameter represents a balance between the magnitudes of vorticity and deformation (Veneziani et al. 2005). Since the Okubo-Weiss parameter typically assumes highly negative values inside coherent vortex cores, while it becomes highly positive in the area immediately surrounding the vortex cores, it is very useful in identifying vortices and rotating structures, like eddies in the ocean. It measures the relative contribution of deformation and vorticity in a velocity field. Taking into account the characteristics of two-dimensional flow fields, eddy cores can be identified as the simply connected regions with high negative values of the Okubo-Weiss parameter. This parameter was used to identify and track eddies from altimeter data (Isern-Fontanet et al. 2003 and Cruz Gomez and Bulgakov 2007).

It is given by $Q = d^2 - \zeta^2$, where ζ is the relative vorticity field which is defined as

$$\zeta = \frac{\partial v}{\partial x} - \frac{\partial u}{\partial y}$$

and d is the deformation rate whose squared value is defined as

$$d^2 = \left(\frac{\partial u}{\partial x} - \frac{\partial v}{\partial y}\right)^2 + \left(\frac{\partial v}{\partial x} + \frac{\partial u}{\partial y}\right)^2$$

A series of 6-hourly maps of Okubo-Weiss parameter for 2004 has been prepared to identify and track eddies in the domain. One eddy (known as the Tortugas Eddy) appears on May 29 and becomes well organized as it moves along the shelf (Fig. 3). Its evolution is shown along with isobaths of 100m and 200m in black lines. Green lines mark 20°C isothermal lines at 150m to capture the Florida Current frontal boundaries (inner and outer). Variability of eddy strength and shape happens within these boundaries. As the eddy moves, it becomes elongated with smaller eddies embedded.

Some of the simulated eddies are captured by SeaWiFS chlorophyll-a images distribution. A big eddy south of Key West is present both in the SeaWiFS chlorophyll-a distribution and the FKEYS simulation. Also a chain of sub-mesoscale eddies along the Keys bounded by the 100m isobath line are shown (Fig. 4).

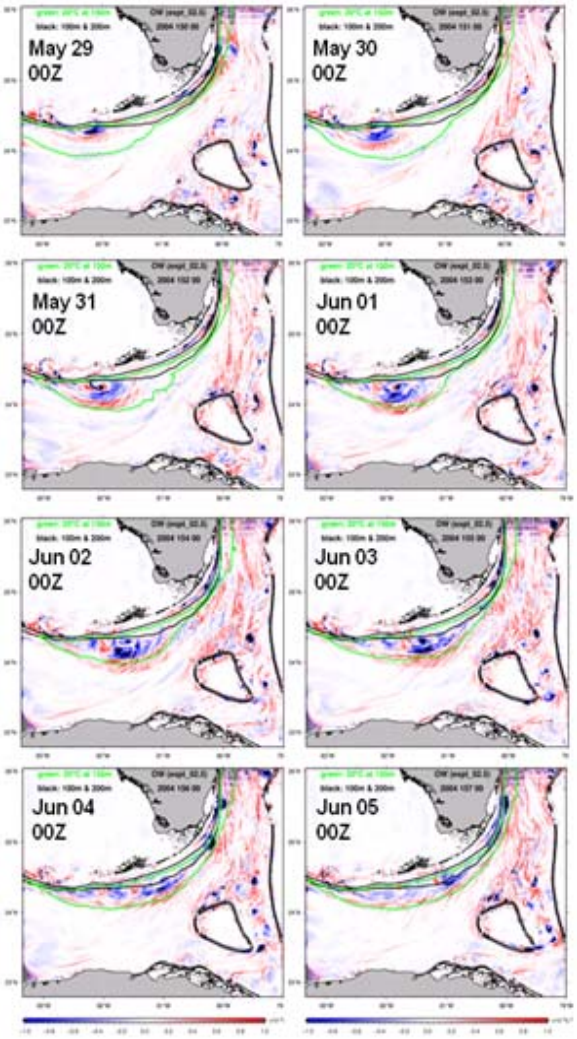

Figure 3. A series of daily Okubo-Weiss parameter maps from May 29 to Jun 5, 2004. Black lines are for 100m and 200m isobaths. Green lines are contour lines of 20C at 150m.

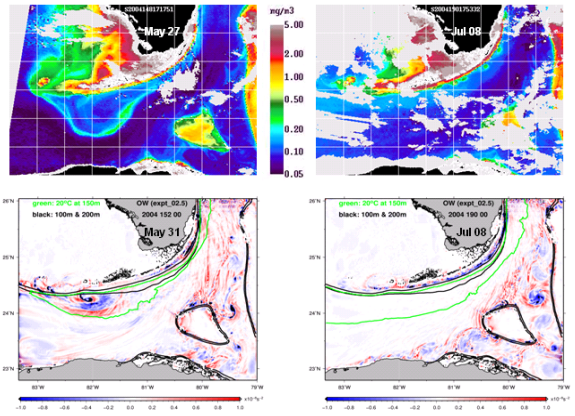

Figure 4. Distributions of Chlorophyll-a on May 27 and Jul 8, 2004 are shown on the upper panels while HYCOM-FKYS simulated Okubo-Weiss parameter maps on May 31 and Jul 8, 2004 on the lower panels.

Eddy induced Upwelling

A mesoscale eddy occurs near the lower part of the Florida Keys Reef Tract (see the top fourth picture in Fig. 5). Cross-sectional distributions of simulated temperature, salinity and along-shore velocity along the western edge of the eddy (82°W) show the upwelling of cool waters, impinging upon the Florida shelf. Strong positive velocities indicate the eastward flowing Florida Current, while velocities turn cyclonically to westward in the vicinity of the eddy.

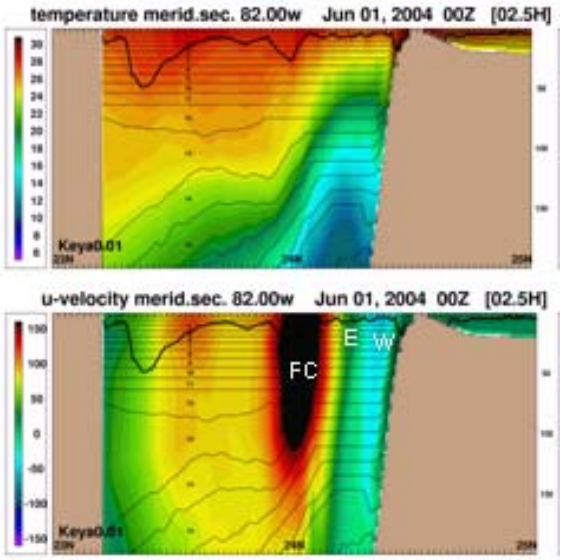

Figure 5. Cross-sectional distributions of simulated Temperature (upper) and along-shore Velocity (lower) during an eddy passage along 82.0°W on June 1, 2004.

Biophysical Lagrangian Tracking System (BOLTS) coupled with FKEYS-HYCOM

Coupling with the ecological population connectivity BOLTS model allows simulations of larval transport, taking into account not only the dispersion of active physical larvae, but also the interaction of factors influencing larval survival, habitat selection and condition at settlement (Paris et al. 2007). This probabilistic modeling system is composed of multiple standalone code units (i.e. biological seascape, ocean general circulation modules) coupled in a single Lagrangian stochastic unit. Of particular interest is the flexibility of the biological module in integrating any type of organism's life history traits, stochastic and spatially explicit larval mortality, larval vertical migration, and larval settlement behavior through the perception of suitable settlement habitat via coupling of the seascape module. In addition, BOLTS runs very efficiently in parallel processing within a LINUX PC Cluster environment and generates various types of output files, including individual larval trajectories and probability transition

matrices of individual larvae migrating from a source to a receiving population.

Passive 30-day near surface advection of larvae released at a single location in the Dry Tortugas computed with the BOLTS model is shown in Fig. 6, where random walk has been added on the FKEYS hydrodynamic model velocity fields. Trajectories and particle distribution integrated over the 30-day period show looping trajectories and accumulation of particles into small eddies (see orange-red particles). This result shows that eddies are an important mechanism of larval transport and delivery.

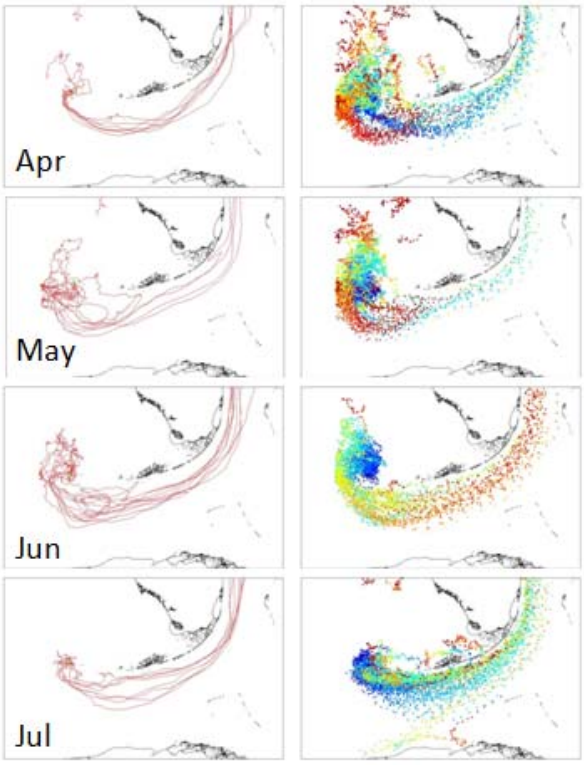

Figure 6. Passive scenario using 6-hour output of the FKEYS-HYCOM 2004: Monthly 30-day trajectories (left – a maximum of 10 trajectories are shown for clarity) and transport of 100 individual particles integrated over 30 days (right) released in the upper 5 m at a single location in the Dry Tortugas Ecological Reserve (green start). Color-code of the particles indicate their age from day 1 (blue) to day 30 (red). Areas with blue and cyan particles indicate strong advection, while areas with concentration of red particles represent retentive flow and/or the end of the 30-day advection.

Acknowledgement

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Use of the Coral-sel Technique in the Study of Small Scale Water Flow Environments on Coral Growth

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Abstract. When flow experiments are carried out in open flow-through systems, several non-targeted variables may also change with varying flow rate, including temperature, oxygen level, and food availability. In order to keep these factors constant across flow treatments, it is necessary to place all corals into a single water system while still exposing them to different flow rates. In order to accomplish this, a rotating "coral-sel" was constructed at the University of Miami's Aplysia Resource Center. The coral-sel allowed corals to experience specific flow rates based on distance from the center of rotation while being immersed in a common water system. Clod card techniques were used in order to test differences in flow rates at each of the specified distances prior to attachment of coral to each position. Fragments of *Montastraea faveolata* were studied during pilot experiments. The coral-sel allowed observations of the effects of water flow on growth, zooxanthellar activity, and morphological characteristics of corallites. Preliminary findings show that water flow alone can influence a coral's growth in terms of surface area, weight gain. Additionally, flow influenced the photosynthetic activity of symbiotic zooxanthellae.

Key words: water flow, coral, growth, photosynthesis.

Introduction

As sessile organisms, the water that surrounds and moves over coral serves a number of important functions that assist in maintaining their existence. These functions include the delivery of dissolved gases and nutrients. Surrounding waters also provide zooplankton and particulate material that the coral may use as sources of heterotrophic nutrition. Additionally, moving water serves to remove harmful chemicals and waste products. Gaining an accurate understanding of the ways in which water flow rates influence coral's health and growth rates will allow for better prediction of the ways that coral, in both natural and artificial habitats, may behave under a variety of flow conditions.

As water moves past an object, a momentum boundary layer forms due to fluid viscosity. The thickness of the boundary layer is inversely related to the speed of water flow past an object (Patterson et al. 1991). The exchange of dissolved materials in and out of the coral is influenced by water motion, largely due to the effects of water motion on the formation and density of this boundary layer and the related influences on diffusion rates of materials through it (Patterson 1992; Patterson and Sebens 1989; Patterson et al. 1991; Bruno and Edmunds 1997; Gardella and Edmunds 1999; Gardella and Edmunds 2001; Kuffner 2001). Water flow rate and its influence on boundary layer thickness and exchange of dissolved materials can influence metabolic rates of coral and the symbiotic zooxanthellae contained within their tissues. Water flow can influence the rates of respiration, photosynthesis, and calcification of corals (Dennison and Barnes 1988). Water acts as a source of nutrition by providing essential chemicals as well as zooplankton for heterotrophic feeding. In this way, water flow can impact coral growth and survival through its influence on the prey capture method and success of coral colonies (Abelson, Miloh et al. 1993; Helmuth and Sebens 1993; Johnson and Sebens 1993; Helmuth et al. 1997; Sebens et al. 1998; Piniak 2002).

The manner in which differing water flow rates and boundary layer thicknesses will influence growth rates and/or morphological development of coral is species specific (Helmuth and Sebens 1993; Bruno and Edmunds 1997; Helmuth, Sebens et al. 1997). In order to accurately determine the influence of the water flow rate and the associated boundary layers on coral growth, it is necessary to reliably isolate water flow as the only variable in an experiment. This is a difficult task using standard laboratory or field methods.

In addition to the effects of water flow experienced under "ordinary" conditions, recent studies have found that increased water flow rates can temper the severity of the damage caused by a variety of stresses on corals. For example, the negative effects of

increased temperatures and photoinhibition can be alleviated by water flow in species and locally specific manners (Jones, Hoegh-Guldberg et al. 1998; Nakamura and van Woesik 2001; Nakamura et al. 2003; Nakamura et al. 2005). Given the current concerns about coral survival under changing climate and water chemistry conditions, gaining a better understanding of any factor that could alleviate stress on these animals may provide valuable information for preservation practices.

Investigating the effects of flow on coral growth under current water chemistry conditions and those predicted to occur in the future, will serve a two-fold purpose. First, by determining the flow conditions that are most conducive to coral growth and survival under current water chemistry conditions, we will be able to create more efficient coral nurseries. These nurseries may in turn serve as a source of coral for both scientific experimentation and reef restoration. This will minimize possible deleterious effects of these activities on natural populations.

Secondly, we may be able to determine the conditions best suited to preserving coral in natural areas under current and possible future water conditions. We would then be able to choose areas that fit these conditions to be set aside as marine protected areas. These protected areas may serve as refugia and possible source populations for corals during the stressful conditions likely to occur in the coming years.

In the past, most flow studies have been carried out either in natural habitats or controlled flumes. In natural habitats, a multitude of factors may vary between study sites. In most flume-based studies of flow effects on coral growth, the partition of treatments into separate systems can be a source of dissimilarity in factors other than the intended study variable. Changing the speed of water movement in an open or flow through flume system may lead to differences in temperature, nutrients, and oxygen levels experienced by each experimental colony. In order to remove this source of error, a research method was needed that allowed all experimental fragments to be exposed to the same conditions, yet still experience different flow speeds.

This paper introduces a new method of research and its possible uses and benefits in flow investigations.

Material and Methods

The method that was devised to reduce the difficulties mentioned above was the "coral-sel". This consists of a rotating structure that can hold a number of corals within a single water tank, but allows the attached corals to experience flow rates that vary based on their distance from the structure's center of rotation

The prototype coral-sel (Fig. 1) was constructed at the University of Miami's Experimental Hatchery and placed in an outdoor circular (3.7 m diameter) tank. The coral-sel consisted of a rotating column with ten arms at five separate heights (each level separated by 15 cm). The positions of the arms around the central axis of rotation were staggered to minimize possible effects of the movement of one plate or arm on a coral at any other position.

Each arm was approximately 95cm in length from the center of rotation, and held three ceramic tiles (experimental units) along its length (Fig 2). Tiles were placed at distances of 15, 50, and 85 cm from the center of rotation. This allowed the experimental units to experience water flow rates of 4.7 +/- 0.5cm/s, 15.7 +/- 1.4 cm/s, and 26.7 +/- 2.4 cm/s when the coral-sel spun at 3 rpm.

A large bicycle wheel was attached to the top of the central rotation column in a horizontal position. The wheel rim was lined with rubber to create friction for the chain that would cause it to turn. A table was positioned along-side of the tank that held a PVC frame attached to a wooden base that held a second bicycle wheel (with tire and gears) horizontally and held on an axle. The frame allowed the wheel to spin freely. A series of bicycle chains were connected to create a single chain long enough to connect the gears on the balanced tire and reach around the outside edge of the rubberized tire on the coral-sel. The frame also held a ½ horsepower motor with the spindle placed in contact with the outside edge of the balanced tire. When the motor was activated, the balanced tire spun at 32 rpm. Use of the attached gears allowed the rotational speed of the coral-sel to be adjusted to the desired 3rpm.

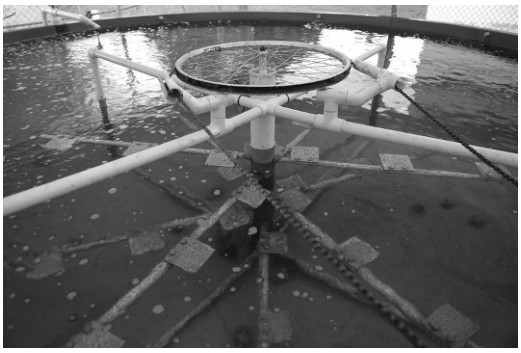

Figure 1: The Coral-sel in position at the UM Aplysia Resource Center. Each attachment site bears experimental plates at positions 15, 50, and 85 cm from the axis of rotation.

Each experimental unit consisted of a ceramic tile (10.8cm x 10.8cm) with limestone blocks (7.6cm x 7.6cm x 0.6cm) secured to its upper (rough) surface. A round (2.5cm diameter x 0.6cm height) plug of live *Montastraea faveolata* was inserted into a matching

hole that had previously been drilled into each square limestone tile (Fig. 2). The plug height was adjusted until it was flush with the surface of the limestone square. Each fragment used in this experiment came from the same parent colony. This colony was relatively flat and had been monitored for over a year in a shallow outdoor tank. Taking all fragments from this single colony maximized the likelihood that variability in responses would be a result of the experimental treatments rather than a result of prior genetic differences, life history, or initial zooxanthellar complement.

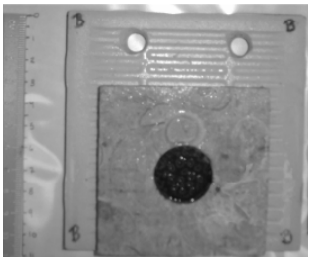

Figure 2: Experimental plate from coral-sel at the initiation of the study. The holes in the ceramic tile allowed attachment to the coral-sel arms with two nylon bolts. The smaller limestone tile is attached using marine sealant. The coral plug sits in a circular hole in the limestone and is attached to the ceramic tile using marine epoxy.

To determine the possible effects of moving the corals in a circular motion compared to passing water linearly over a stationary coral, controls using submersible pumps were tested. Two submersible pumps were each plumbed to allow four nozzles to pump water over stationary coral plates at speeds equal to those experienced by corals 50 cm from the coral-sel's axis of rotation (15.7cm/s). Growth experienced by corals placed in the "pump" treatments could then be compared to that experienced by those in coral-sel treatments.

After construction, and prior to the beginning of experiments, the flow rates at each position were examined using the clod card technique to determine if flow rates at those sites were equal at equivalent positions on the coral-sel (i.e. all 85 cm positions experienced equal flow). This method was also used to verify that the flow rates that the pump platform positions experienced were equal to those experienced by the experimental units in the medium flow treatment on the coral-sel.

All experimental units were removed from the coral-sel bi-weekly. Each was cleaned using a small brush to remove any sediment and algae that had accumulated on the tile. Care was taken when removing material close to the coral in order to minimize the possibility of damaging new coral growth.

After cleaning, excess water was removed from the experimental units using paper towels. The corals themselves were lightly "dabbed" dry, but no pressure was applied. This was to avoid inflicting damage on the delicate coral and skeleton.

Each plate was then weighed on an electronic top loading balance. The weight of each original unit (minus the coral) was subtracted from the measured weight in order to determine the true weight of the fragment at the time of weighing. Weights of coral fragments under different flow conditions were compared statistically using ANOVA.

Each experimental unit, along with a standard metric ruler, and color standard was then photographed using a digital camera. Each photograph was analyzed using the Image J computer program. Using the program, the perimeter of each fragment's surface was traced, and the surface area calculated. The surface areas of fragments were compared statistically using ANOVA.

Results

The experiment ran for a total of 18 months, but three months was adequate to observe significant differences in growth rates based on flow rate (Fig. 3). These preliminary findings showed that flow rate caused significant differences in surface area growth ($p=0.0051$). The lowest flow speed yielded the greatest increase in surface area and the highest flow speed yielded the lowest. The change in area exhibited by the "control" corals placed in front of the pumps, did not vary significantly from that of the corals placed under an equivalent flow rate on the coral-sel ($p= 0.5757$).

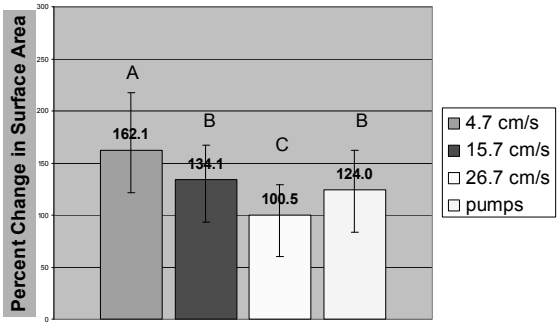

Figure 3: Average percent change in surface area of fragments after sixteen weeks (9/5/2005 – 12/26/2005) . Groups labeled with the same capital letter are not significantly different. ($p = 0.5757$).

Corals experiencing 15.7cm/s of flow showed the highest weight gain over the same period (Fig. 4). Therefore, under these conditions, weight gain was not necessarily dependent on increase in area. In fact, during these early weeks of the experiments when weight increases were standardized to increases in area, the greatest weight/unit area increase was seen

in the high flow speed treatments, though this pattern was altered later in the study.

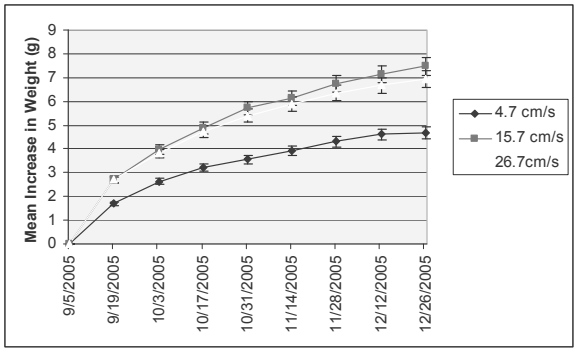

Figure 4: Mean cumulative weight gain over a sixteen week period.

In order to determine whether the water flow rate over a coral fragment influenced the photosynthetic abilities of the zooxanthellae, the electron transtport rates of the zooxanthellae contained in each fragment were calculated using the light curve program of a Walz Diving PAM fluorometer. The fluorometer produced a series of light pulses of increasing intensity, and the fluorescence of the zooxanthellae in response to each pulse was measured and recorded. These values were used to calculate response curves similar to those seen in Fig. 5. Measurements were taken in the morning hours (approximately 0800 to 0930 local time). The ETR curves produced using this method were similar to those seen in figure 5. In general, the maximum electron transport rates of corals on the coral-sel were seen in fragments experiencing the highest flow rates. Under the experimental conditions, the P_{max} level appears to be directly related to the flow rate.

Further examination of the light curves for all fragments reveals that the minimum saturating irradiance for zooxanthellae in these fragments show the same inverse relationship to water flow rate.

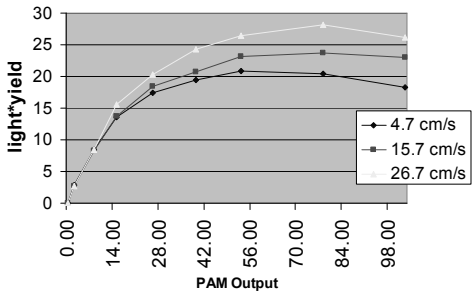

Figure 5: P-I curves exhibited by corals as a function of flow rate.

Discussion

The movement of water over a coral colony can influence its growth and survival through effects on boundary layer thickness. The boundary layer in turn influences polyp movement and prey capture ability (Sebens *et al.* 1998), gas, nutrient, and waste exchange rates, as well as products used in metabolic activities (Kuffner 2001). In addition, flow has been seen to influence the accumulation of mycosporine like amino acids (MAAs) within coral tissues (Jokiel et al. 1997, Kuffner 2001).

While flow has been shown to influence each of the above factors, previous methods of studying flow effects both in the field and in flume systems were unable to completely isolate flow rate itself as an experimental variable. In flume studies, corals experiencing different water flow rates are placed in complete isolation from one another, and water being used to create the flow rates does not mix. This may lead to additional differences in the treatments that vary due to flow, such as temperature and oxygen levels. In the field, corals in areas with different flow rates may also experience different nutrient and food availability conditions.

These confounding factors have made flow as an experimental variable exceedingly difficult to isolate for examination. The solution to this difficulty explored in this study was the "coral-sel method" which allowed fragments of coral to be placed in a common fluid environment, yet still experience different flow rates. The finding that growth on the coral-sel did not vary significantly from growth by corals under similar flow rate conditions in a stationary position add validity to this process as a method to investigate water flow influences on coral.

The coral-sel method allowed water flow effects to be examined with confidence that all other factors, including temperature, light availability, oxygen levels etc. remained equal for corals in all flow treatments. Examination of the corals on a biweekly basis allowed monitoring of changes in coral fragment surface area, weight, and photosynthetic ability. Upon completion of the study, the fragments can be examined to determine whether they have experienced a change in zooxanthellar complement through use of genetic methods. In addition, the fragments can be examined to determine if basic corallite structure changed based on water flow rate alone.

Acknowledgements

I first thank Rob Carter who helped in the original design and construction of the coral-sel prototype. I would like to thank my academic advisor Peter Glynn for his support and advice. In addition, I thank Chris Langdon, Andrew Baker, Daniel DiResta, Robert van Woesik, and Su Sponaugle for their guidance and recommendations throughout the design and experimental phase of

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Mini-Symposium 15

ASTER bathymetry in computational fluid dynamic simulation of Rongelap Atoll hydrodynamics, Marshall Islands

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Abstract. ASTER green, red and near infra-red (NIR) imagery with a resolution on the order of handheld GPS echo soundings was calibrated to model bathymetry of Rongelap Atoll to a depth of 10 meters. The ASTER model was converted into of depth contours at 1, 2, 4, 8, and 16 meters. Beyond that depth nautical charts and echo soundings were used to model bathymetry. The combined result was a bathymetric digital elevation model of Rongelap Atoll and surrounding seas, which was converted to X,Y,Z file and for input into reverse engineering software. The reverse engineering software then created a NURBS (Non-Uniform Rational B-Spline) surface model, for input into the finite element meshing program GAMBIT (ANSYS Fluid Dynamics International). These data were then ordered in volumetric and substrate surface elements of coral reefs and input to the FLUENT computational fluid dynamics (CFD) package. Bluelink (Australian Bureau of Meteorology and CSIRO) was used to apply boundary conditions, and results are hydrodynamic charts of the atoll. The resulting map of substrate shear stress at Rongelap is displayed in Figure 2. Figure 2 Benthic shear stress of Rongelap Atoll.

Key words: Atoll, Hydrodynamics, Remote Sensing.

Introduction

Peterson, et al. (2005) argued that computational fluid dynamics are needed to classify regions of benthic shear stress in coral reef ecosystems. That study also presented correlated biological and physical observations from Rongelap Atoll to demonstrate the importance of benthic shear stress in defining habitat suitability in coral reef ecosystems.

The present paper seeks to map Rongelap's previously uncharted waters and to improve our understanding of hydrodynamics in, through, and around the vicinity of this atoll.

Four different datasets were fused to create the digital elevation model employed in the present paper with the ultimate goal to simulate the hydrodynamics of Rongelap Atoll. A nautical chart (Fig. 1) was available, which was copied from Japanese Navy surveys 1917 and 1922 under League of Nations Mandate, and since reprinted by the US government. This chart only covers a fraction of the atoll, and the coordinates of this chart are not WGS84. Likely the original surveys were based on sextant placement of a granite monument (with Japanese characters) found in the main settlement of Rongelap. To this day numerous digital charting products have misplaced every single feature of Rongelap Atoll by at least a mile, but Reston (2007) used the ASTER scene to adjust the chart with WGS84 graticule marks.

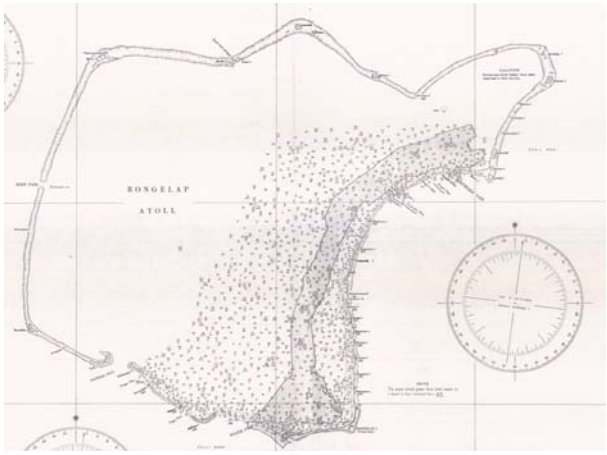

Figure 1: Latest published chart of Rongelap (evidently a copy of Japanese surveys 1917 and 1922, Alele Public Library, Majuro).

Another bathymetric dataset available were coincident GPS tracklogs and echo-sounding during reef surveys (Pinca et al. 2002; Peterson et al. 2005). This largely filled in the western side of Rongelap lagoon, but failed to reach the far northeast corner.

The deepwater bathymetric dataset used in the present study was from Hein et al. (2007). But parts of Rongelap were not covered by the digital elevation model, and little information exists on shallow waters that flood over reef crests. Reefs are by their nature too shallow for navigation, and so not fathomed on nautical charts. Thus, remote sensing is a useful tool to estimate depth. We used an ASTER scene, of which we excluded the blue (B) band, but included the green (G), red (R) and near infrared (NIR) for determination of the land/water interface.

Bathymetric modelling utilises a variety of techniques comparing green and blue wavelengths (Reston 2007). For the present study, visible green only was used for bathymetric calculation, and as a consequence, was expected to yield results with lesser depth penetration than those expected with the inclusion of visible blue data.

Reston (2007) constructed a linear regression model using sub-samples of validation data derived from GPS tracked echo soundings (Pinca, et al. 2002, and Peterson 2005), and historic nautical charts to predict depth from the calibrated ASTER data. Land and breaking water was identified using the NIR band, and assigned a threshold value of 0.5m to preclude their inclusion in the bathymetric calculation, while bathymetry deeper than 20m was assigned that value as a constant. Depth penetration of green light in the ASTER Rongelap scene is approximately 20 metres, exceeding expectations, owing to the clarity and optical penetrative characteristics of the Marshall Islands waters.

An example of results from the southeast corner of the ASTER scene is detailed in Figures 1 and 2, being the main island of Rongelap.

Approximately 15% cloud coverage exists in the NE quadrant of the ASTER image. These have been masked by manually editing contours extracted from the pseudocolour water depth DEM

Material and Methods (CFD modelling)

The ASTER-derived contours of shallow water bathymetry were then kriged together with the nautical chart soundings, NRAS survey track logs and the deep water regional bathymetry to produce a continuous DEM seafloor surface of the Rongelap Atoll region as an ASCII Raster file with 300 meter resolution, and then converted into x,y,z point-cloud data in comma separated values (CSV). The seafloor DEM as CSV was then converted into NURBS (Non-Uniform Rational B-Spline, with algorithms of Bézier & de Cateljau (Farin 2002) surface using ProEngineer "reverse engineering" toolbox (Fig. 4).

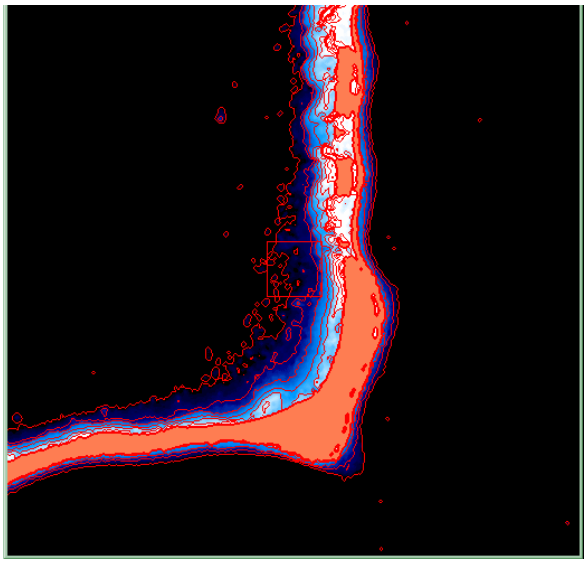

Figure 2: False colour water depth overlain by 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 m contours in SE corner of Rongelap Atoll.

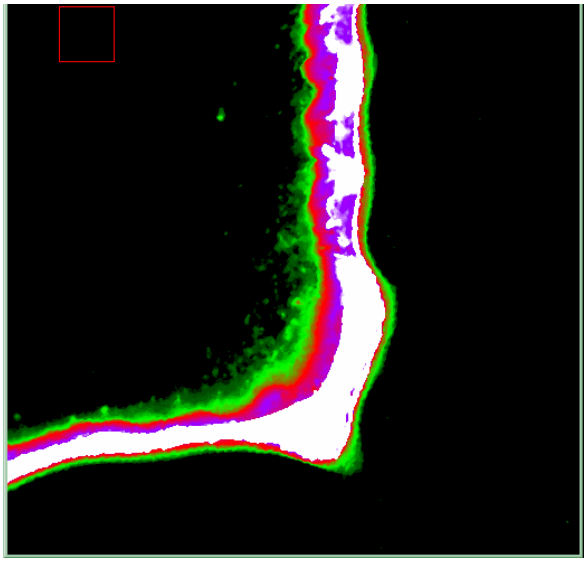

Figure 3: Pseudocolour water depth DEM product in SE corner.

The NURBS surface represents the complex seascape of coral reefs bounding the lower face of the fluid flow problem, while the upper surface is given a fixed-lid approximation as determined by sea level as the air/water interface. This modelling approach suits extensive submergent reefs such as Micronesia with only "small islands" set in trade wind circulated seas. The NURBS surface was saved in IGES format and then imported into the meshing program GAMBIT (Figure 5) for development of the CFD domain.

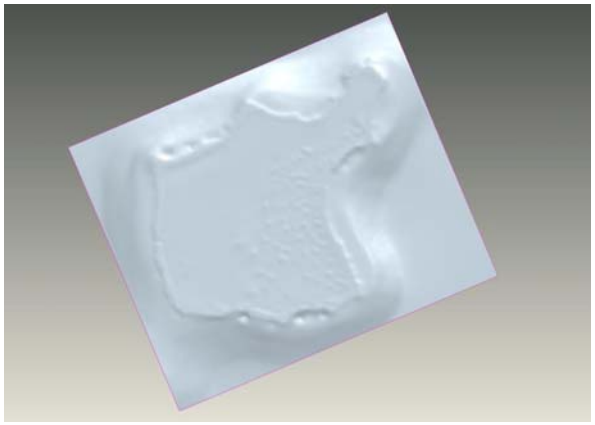

Figure 4: Screenshot from ProEngineer of Rongelap NURBS.

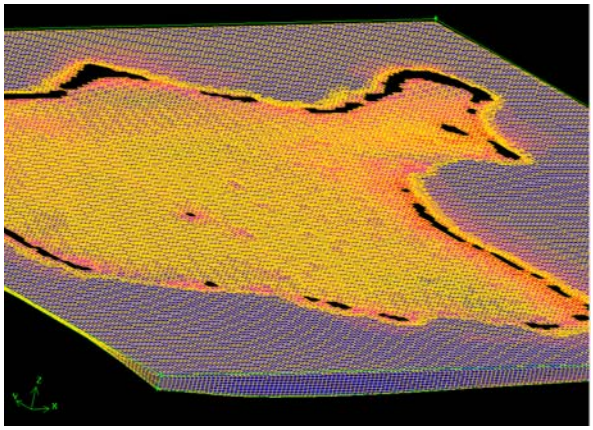

Figure 5: Screenshot from GAMBIT mesh generation for Rongelap model. Slicing ten meters into the domain, islands and reef flats appear as voids, but there is flow allowed to go over all areas.

The computation mesh was then read into Fluent software with the imposition of boundary conditions referencing the BLUELink conditions of Figure 6:

- Sea surface – wall allowed to slip with specified wind shear 10 knot (5 m/s) tradewind driven current flow east>west stress vector (1,0,0) [Pa]
- Sea floor – no-slip wall and with shear stress reactions evaluated by CFD
- Sea to north, south, and west – Current from east (-.2,0,0) [m/s] velocities from BLUELink (www.bom.gov.au).
- Sea to west – low pressure exhaust [-100Pa].
- turbulent intensity ~30% of mean flow based on BLUELink> 1st-14th Jan 2000.
- hydraulic radius ~ 1000m depth of ocean.

BLUELink> is described by Brassington (2007).

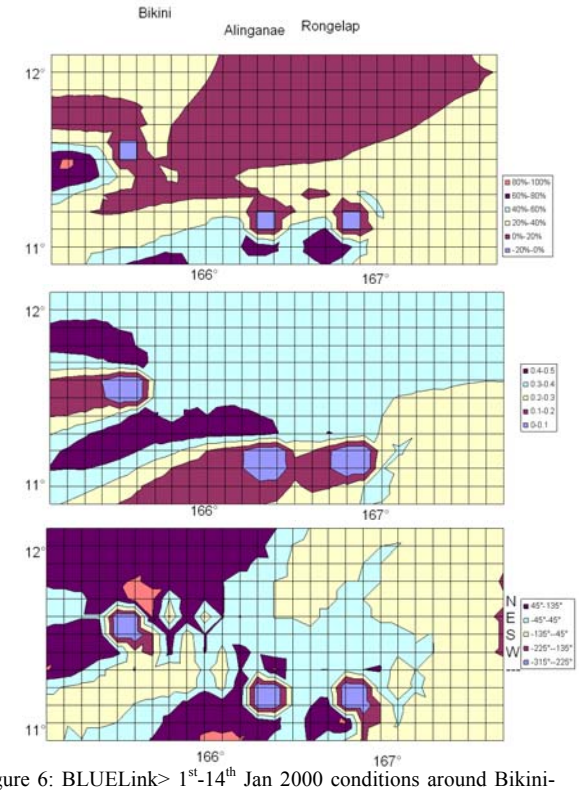

Figure 6: BLUELink> 1st-14th Jan 2000 conditions around Bikini-Alinganae-Rongelap region. Turbulent Intensity above, mean speed middle, and direction below.

The turbulent viscosity model is realizable k-epsilon with the following features:

- Turbulent intensity $I = u'/\bar{U}$ = rms/speed
- Eddy scale ~ 7% of hydraulic diameter
- Turbulent kinetic energy $k = {}^{3}/_{2}\cdot(\text{speed}\cdot I)^2$
- Turbulent dissipation rate $\varepsilon = 2.35\ k^{3}/_{2}/L$
- Turbulent viscosity $\mu_t = 0.09\ \rho\ k^2/\varepsilon$

Results

Preliminary benthic shear stress results of simulation are illustrated in Fig. 7. The strong red spot on the main island of Rongelap is fictional, since this island is not actually covered by the present sea level. This result could be masked out with polygons of land, to provide a Geographical Information System product of the CFD simulation.

Other important products of the CFD model could be the larval connectivity matrix between each reef, but this has not yet been produced for Rongelap Atoll.

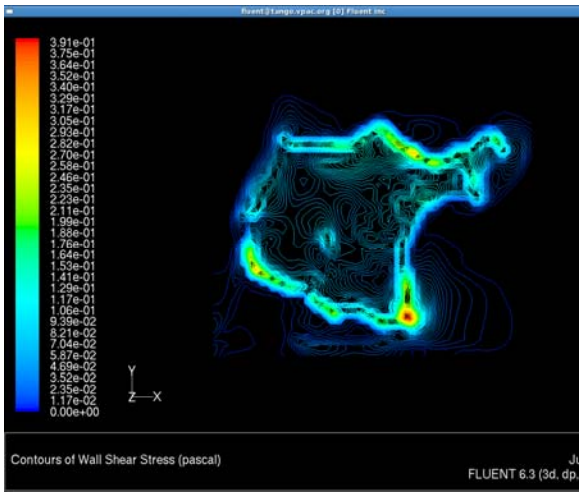

Figure 7: Resulting benthic reaction [Pa].

Discussion

BLUELink> provides real-time 11km resolution hydrodynamic conditions throughout the Indo-Pacific. This provides an opportunity to model local areas of interest, such as Rongelap Atoll. BLUELink> provides seaward and airside boundary conditions to CFD modelers. It is then a problem of nesting mesoscale models within the regional model provided by BLUELink.

Convergence has generally improved in solutions where u,v,w components of flow are specifically mapped at the seaward boundaries of the CFD model. It has proven better to also define the western seaward boundary with explicit velocity components rather than an exhaust condition.

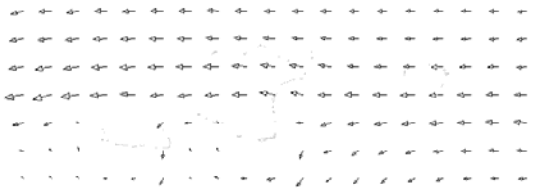
Figure 8: BLUELink> surface vectors around Alinganae and Rongelap Atolls 1st January 2000. Many layers of the ocean, and many years of daily data are available by automated web queries.

The DEM will be extended around Rongelap to match the BLUELink grid illustrated in Figure 8. The DEM model of Hein et al. (2007) extends well into these deeper waters, and so it will not be difficult to resample it to produce a larger NURBS domain. For hydrodynamic modeling of the Rongelap region, the hypothesis is that the critical factor to determine flow through atoll systems is the shallowness of the reef flats, where red and green light penetration is amply suited to measure bathymetry. Figure 9 illustrates the thin sheet of fast moving flow over a reef flat.


Figure 9: Flow over Rongelap reef flat, particle tracked with GPS.

The CFD model is yet to be validated with the GPS tracklogs illustrated in Figure 9. In that particular fieldwork exercise a tide guage was set up to establish a staging-curve of flow over the reef flat. An echo-sounder provided the depth of water, while the gps measure the velocity of surface water flow of a snorkler following slightly positively buoyant floater illustrated in Figure 10. Thus each tracklog has a coincident depth log, and so the two integrate volumetric flow over the reef flat per cross unit width.


Figure 10: Flow over reef flat, tracked with echo-sounding GPS

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Reef Rip Current Generated by Tide and Wave during Summer Season: Field Observation Conducted in Yoshiwara Coast, Ishigakijima, Okinawa, Japan

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Abstract. In 2004 and 2005, five drowning accidents in Ishigakijima, Okinawa were documented by Japan Coast Guard and were found to be caused by a strong offshore current commonly known as "reef current". This type of current has been identified to be responsible in generating the circulation of water and transportation of sediment in coral reefs. In this paper, however, high-speed offshore current in coral reefs is specifically referred to as "reef rip current". Considering that the generation mechanism of reef rip current is not revealed yet, the researchers conducted a hydrodynamic study in Yoshiwara Coast, Ishigakijima, Okinawa in two phases (normal and storm conditions) during summer season to determine the location and time of occurrence of the strong offshore current that will significantly address safe utilization of coral reef and carbonate beach thereby reducing drowning accidents. The nearshore hydrodynamic project that was undertaken included field measurement of wave, current and tide during normal and storm conditions with the implementation of bathymetry survey by laser method. The results revealed the maximum 20-minute average offshore velocity at 1.2m/s. In addition, it was observed during low tide condition that most of the water inside the lagoon is only discharged through the reef gap.

Key words: Reef rip current, tide and wave dominated, coral reef, safe utilization, water circulation

Introduction

Coastal areas in tropical and subtropical regions are characterized by coral and normal sandy beaches that are attractive for tourists around the world thus making beach and marine tourism one of the fast growing economic industries. On the other hand, utilization of beaches also poses a risk on the safety of the coastal users who are not familiar of nearshore current system. Hence, field study and analysis of drowning accidents in carbonate beach and coral reef have been implemented and conducted in Japan's subtropical region, Okinawa Prefecture particularly in Yoshiwara coast, Ishigakijima in order to establish and describe the strong offshore current mechanism.

Monismith (2007), Kench et al. (2006), Tamura et al (2006), Hearn (2001, 1999), Nadaoka et al. (2001), Yamano et al. (1998), Kraines et al (1998), Symonds et al. (1995), Gourlay (1994), Young (1989), Roberts et al. (1975) among others have studied and made scientific publications on hydrodynamics in coral reefs in tropical and subtropical regions. However, the impact of nearshore current on safe utilization of carbonate beach and coral reef has not been fully established yet. Literatures have revealed that circulation in reef systems is typically driven by a combination of tidal flow, wind-driven flow (Yamano et al. 1998) and flow induced by the breaking of wind-waves on the reef flat (Kraines et al. 1998; Symonds et al. 1995; Hearn and Parker 1988; Nadaoka et al. 2001) and that currents and deepwater waves are significantly modified by reef morphology (Roberts et al. 1975) in a consistent manner dependent on tidal elevation, reef elevation, and reef width (Kench et al. 2006). Currents on the fore-reef shelf exhibit a distinct periodicity near the diurnal tidal frequency wherein the characteristic of the deep (21m) shelf margin is rather unidirectional (Roberts et al. 1975; Kraines et al. 1998) and high-velocity flow (>50cm/s), but on the shallow shelf, a weaker (5-7cm/s) and more

directionally variable currents are found (Roberts et al. 1975). During a typhoon, abrupt decrease and increase of the water temperature with a resultant of about 10C lower than before was documented in the study by Nadaoka et al. (2001). The numerical simulation of Tamura et al. (2006) which showed good agreement with the observed data revealed that currents have an appreciable magnitude of tide-averaged velocities, even during neap tides, which are governed mostly by wave set-up effects.

In this study, however, the relationship and impacts of nearshore hydrodynamics to safe utilization of carbonate beach and coral reef are the key issues.

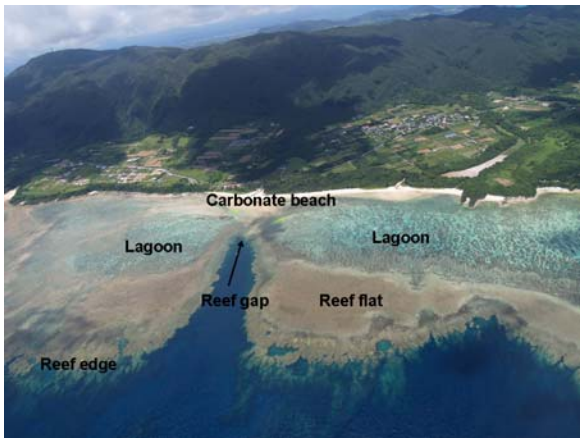

Figure. 1. Aerial view of study site in Yoshiwara Coast

Materials and Methods

A nearshore hydrodynamic project was carried out in Yoshiwara, Ishigakijima's carbonate beach and coral reef coast (Fig. 1) in two phases; Phase 1 was performed during normal condition (small wave and tide dominated) and Phase 2 during storm condition (high wave and tide dominated). The project was implemented in summer season from June 25 to July 25, 2006, the period when the occurrence of drowning accidents had been reported.

An aerial laser survey was conducted for two days by an airborne team from the Sixth Region of Coast Guard in Hiroshima on the actual study site to produce the bathymetry map with high resolution (Fig. 2).

Parameters such as water level, wave height, wave period, water temperature, current and wind speed and direction both in Phase 1 (June 25-July 1, 2006) and Phase 2 (July 1-25, 2006) conditions were obtained through field measurement with the aid of field equipment such as wave gages, electronic current meters (ECM), ADCP, tide gages, thermometer, GPS floats, and anemometer as well as fluorescent dye experiment. These data parameters were analyzed to describe further the wave, tide and current patterns occurring and behaving in coral reef and carbonate beach and consequently to establish their respective relationship.

Results and Discussion

Morphology

The aerial laser survey that produced the bathymetry map in Fig. 2 shows a developed reef flat and lagoon system. The offshore length of the reef system is more than 1,000 meters. Profiles of the reef gap area and the well-developed reef flat and lagoon indicated by transect lines 1 and 2 respectively are shown in Fig. 3. The cross-section along transect line 1 passing thru the reef gap area spans a 250m length of horizontal flat and gradually drops to a depth more than 40m. However, the well-developed cross-section of the reef flat and lagoon indicated by transect line 2 extends a 920m horizontal flat and abruptly drops to a depth more than 40m. Carbonate beach is also developed at the mouth of the river and reef gap boundary. There are also narrow channels that are directed toward the reef gap.

Nearshore wave and current are governed by hard bottom reef geomorphology. In general, incident high wave dissipates its energy over a reef edge and flat depending also on tidal level, i.e., the ratio of waterdepth to wave height. The current in reef lagoon may become weaker but will be faster near the reef gap (locally called "mouth of the coral reef" in Okinawa) which is the location where there is significant geomorphological feature to control the current system inside a reef. Geomorphology of coral reef is the fundamental information to estimate wave and current system, and to justify its safe utilization.

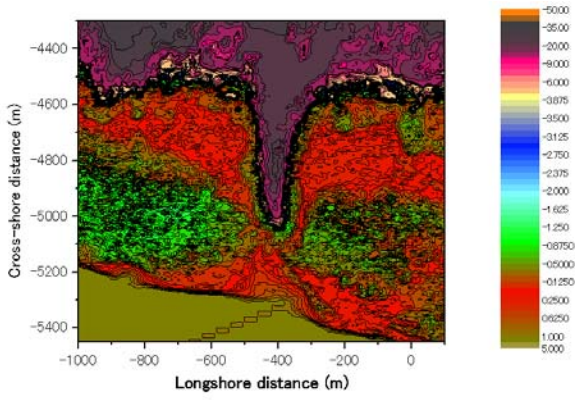

Figure 2. Bathymetry of study area (Yoshiwara coast, Ishigaki, Okinawa Prefecture)

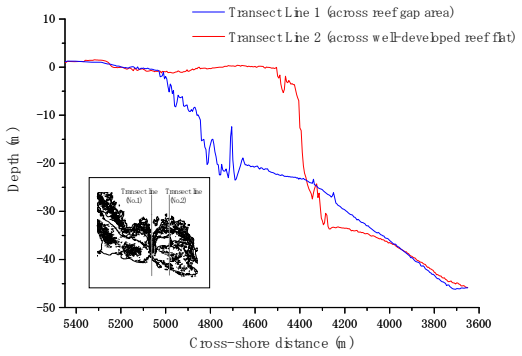

Figure 3. Cross-shore profile of the study area at the reef gap and reef flat (Yoshiwara coast, Ishigaki, Okinawa Prefecture)

Flow visualization by dye and GPS floats

Nearshore current system can be visualized with the aid of dye experiment and GPS floats. Figure 4 shows the point of application of the dye (Pt. A) and its diffusion pattern. The width of the dye is an indication of the strength of the offshore flow which increases in speed as it approaches the reef gap (Pt. B, about 250m in length). As offshore flow moves out from the reef gap, its speed starts to slow down as the flow continues to pass through a much wider cross-sectional area (Pt. C). This is visually represented by the diffusion of the dye which also describes the flow pattern direction.

Figure 5 also shows the flow pattern inside and outside of coral reefs, especially around the reef gap. Small GPS floats originally developed for a study on rip current on sandy beach by Nishi et al. (2003) were applied. The GPS floats are intended to provide direction flows of major and minor currents starting from the points of application from the sandy beach through the reef gap and offshore as represented by Path Lines 1-5 (Fig. 5). Another direction flow mechanism was also applied in conjunction with the GPS floats by a drifter having a GPS gadget that moved in the direction of the current flow (Fig. 5, labeled as staff with GPS) to verify and substantiate flow pattern characteristics and measurements converging through and diverging from the reef gap.

Velocity of a reef rip current

One week duration of nearshore hydrodynamic observation was first conducted from June 25 to July 1, 2006. Figure 6a shows the change in water level inside the lagoon. The tide shows semidiurnal variations. The maximum water level reached up to 2.4m and minimum water level at 0.8m with a tidal difference of 1.6m. The second observation was conducted from July 1-25, 2006 in the presence of storms where maximum and minimum water levels were recorded at 2.5m and 0.75m respectively (Fig. 6b) which resulted into a slightly higher tidal difference at 1.75m.

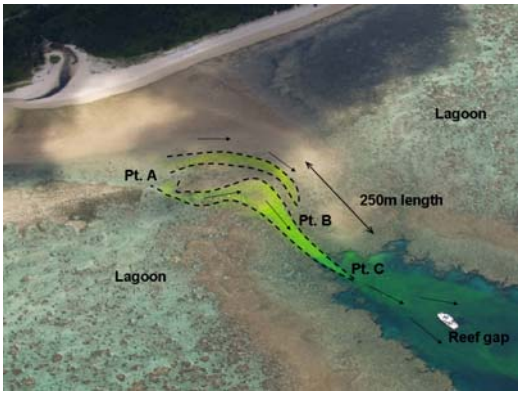

Figure 4. Dye experiment (dye pattern shows an offshore current toward a reef gap)

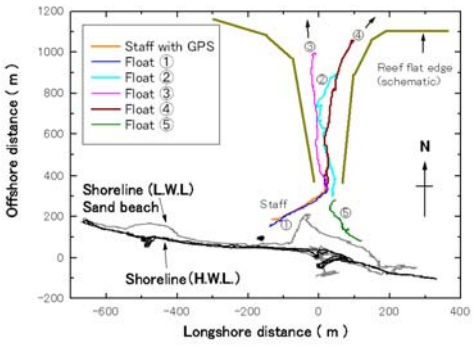

Figure 5. Offshore flow pattern starts from the carbonate beach and moves outside the coral reef through the reef gap

The 20-minute average velocity record in the narrow channel near the reef gap (Fig. 7a) indicates high offshore velocity up to 1.2m/s at ebb tide and reasonably high onshore velocity up to nearly 0.7m/s at flood tide during low tide conditions. The reason for this is attributed to reef edge and reef gap which entrap the water inside the lagoon and outside the reef system respectively. This scenario is similar to a hydraulic dam especially when the water level inside the lagoon becomes lower than the elevation of the crest of the reef flat. At this point, most of the entrapped water inside the lagoon would be concentrated and discharged through a reef gap. Moreover, when the tide changes into flood tide, the crest of the reef flat also has the function to be a barrier against flood tide when the water level is lower than the crest of the reef. Thus, the flood tide (onshore current) is also concentrated into the reef gap. The first and

second peaks of 20-minute average velocity correspond to offshore current and onshore current respectively. Storm condition also forced and triggered the generation of wave height nearly 1.0m at the shoreline. Given this magnitude, it is expected that a much stronger offshore current will yield. However, for safety and precautionary reasons, the equipment had to be relocated to a less risky position which then measured the maximum velocity at 0.25m/s (Fig. 7b). Based on water level and current velocity data, an inverse proportionality relationship has been established as shown in Fig. 8. It is evident that the existence of high-speed current at the reef gap occurs during low tide condition. This scenario has been verified by Japan Coast Guard and local people as to the period when the drowning accidents occurred in Ishigakijima, Okinawa (Nishi et al. 2007).

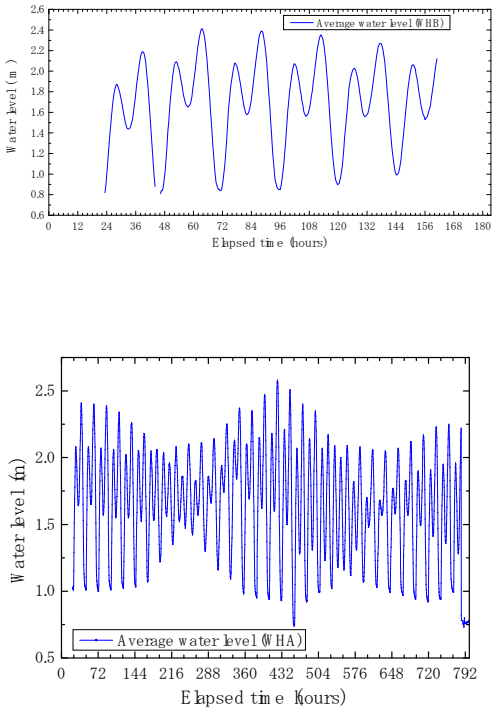

Figure 6. Tide record at the study site (upper panel) normal condition (June 25 – July 1, 2006), (lower panel) storm condition (July 1 – July 25, 2006)

Mean water level

Mean water levels inside and outside of coral reef produce spatial gradients which enhance the flow around a reef gap. To reveal the mechanism of strong offshore and onshore currents through a reef gap, four water level gages were installed on the hard sea bed. The installation points are shown in Fig. 9, one located outside the coral reef at the reef gap, another one on the reef flat near the reef edge, one in the middle of the lagoon, and one in front of the carbonate beach. The water level was measured every 1 second and expected to measure and run up to 11 days. However, due to some mechanical problems, data were stored for the first 36 hours during the first fieldwork. Superposition of four mean water level data is shown in Fig. 10.

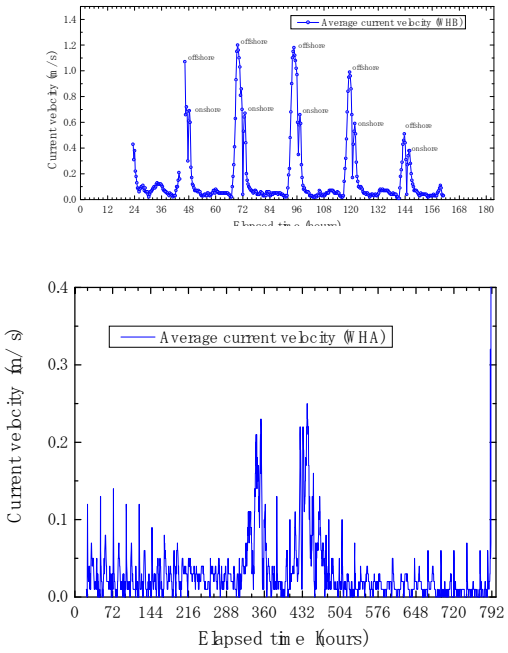

Figure 7. 20-minute mean velocity record (upper panel) normal condition (June 25 – July 1, 2006) (lower panel) storm condition (July 1 – July 25, 2006)

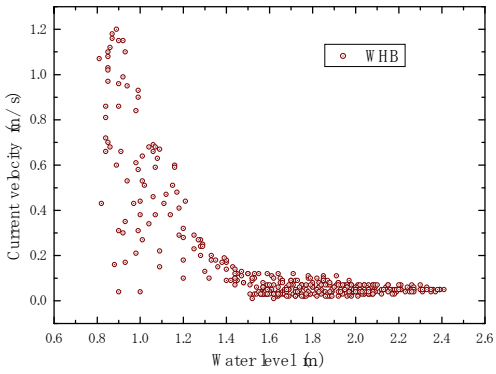

Figure 8. Relationship between current velocity and water level

Furthermore, it has been established that the spatial difference in mean water level is indeed significant especially during the lowest low tide. From the

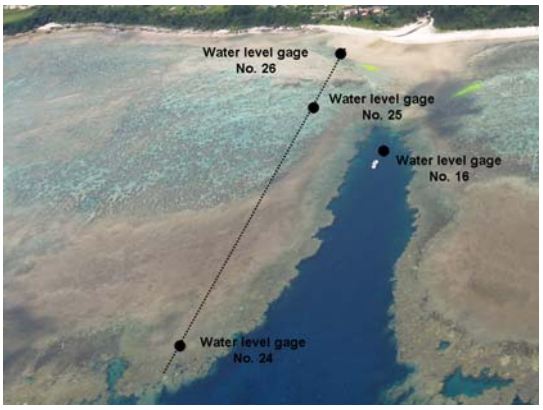

Figure 9. Orientation of water level gages (Gage 16 at the reef gap, Gage 26 on the carbonate beach, Gage 25 in the reef lagoon, and Gage 24 on the reef edge)

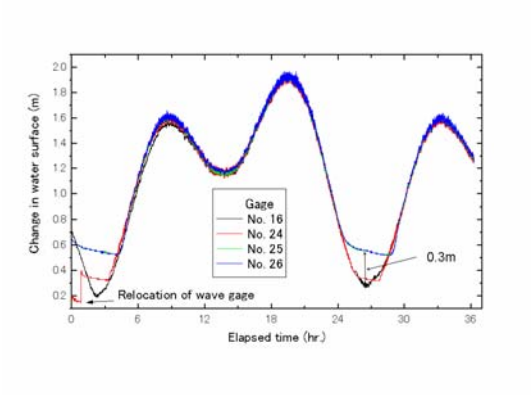

Figure 10. Mean water level outside of coral reef (No.16), on reef edge (No. 24), in the middle of lagoon (No.25), in front of carbonate beach (No.26)

field measurement, it was revealed that the mean water level inside a coral reef was higher than the mean water level outside of coral reef by as much as 0.3m (Fig. 10). Translating this gradient in terms of velocity expressed by $v = \sqrt{2gh}$, where g represents gravitational acceleration and h is the difference in water head, results into a rough estimation of 2.4m/s that is in fact twice as much as that from the observed data. This clearly shows that a spatial gradient of 0.3m would generate a strong current by more than 1m/s at the reef gap which is considered to be an upper limit of coastal users' (swimmers, etc) workability in water specifically in Japan.

Conclusion

The nearshore hydrodynamic study described the reef rip current phenomenon generated by tide and wave has revealed the following;

(1) During low tide conditions, the maximum 20-minute average velocity was measured as much as 1.2m/s even though the significant wave height is small (0.12m).

(2) The reef gap has a function to control exchange of water mass inside the coral reef especially during low tide. The reef flat works as a boundary to entrap the water in the lagoon and allows it to be discharged through the reef gap at low tide.

(3) A resultant spatial gradient in the mean water level inside and outside of coral reef is one of the most important mechanisms of the existence of a strong reef rip current.

Acknowledgement

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Using cellular diagnostics to link land-based sources of pollution with coral reef degradation in South Florida

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Abstract. Linkages between land-based sources of pollution and coral reef health were assessed at ecological, physiological and cellular levels at four paired inshore and offshore stations off Broward County, Florida, U.S.A.: a biomonitoring control site, a treated wastewater outfall, an inlet mouth, and a treated wastewater outfall adjacent to an inlet mouth. Live coral cover was <4% at all sites and most inshore sites clustered together because they had less bare substrate and considerable cyanobacteria (*Lyngbya* sp.). Cellular diagnostics revealed that the stony coral *Porites astreoides* at all sites was stressed compared to colonies from a more remote Bahamian site. Offshore corals exhibited higher biomarker accumulations than inshore colonies. Corals near ocean outfalls and from protected areas in the Florida Keys had diagnostic profiles consistent with treated wastewater exposure. Profiles of offshore colonies were consistent with xenobiotic detoxification. Corals regenerated from sampling lesions at the two offshore biomonitoring control sites and two other inshore sites. Regeneration rates at offshore sites near the offshore ocean outfall and shipping channel were negative. Congruence between ecological, physiological and molecular information demonstrates that using multiple bioindicators can identify linkages between land-based sources of pollution and distressed coral reefs.

Key words: biomarkers, *Porites astreoides*, coral reef health, treated wastewater

Introduction

Land-based sources of pollution are critical factors influencing the fate of coral reefs off the heavily populated southeast Florida coast. Here, we describe a project that integrates traditional monitoring methods with new bioindicator technology – cellular diagnostics – to address how treated wastewater discharge and shipping channels affect coral reefs.

Cellular diagnostics was designed to assess the condition of reef-building corals and identify mechanisms of coral pathologies (Downs 2005 and references therein). It works because environmental stressors affect organisms by overwhelming defenses at lower levels of the biological hierarchy: molecular, cellular, and organismal-level homeostatic processes. By evaluating coral responses at these and higher levels of the biological hierarchy, scientists can provide resource managers with critical information needed to identify and ameliorate stressors before an ecosystem-scale crisis occurs (Fauth et al. 2003)

Methods

Sampling occurred at four paired, inshore and offshore stations located near a treated wastewater outfall, an inlet mouth, a treated wastewater outfall located within an inlet mouth, and a biomonitoring control site off Broward Country, Florida, USA (Table 1). The mustard hill coral (*Porites astreoides* Lamarck) was chosen as the focal species because it is distributed across the shelf in south Florida and is amenable to cellular-diagnostic analyses.

Cellular Diagnostics

Diagnostic antibodies included assays for oxidative stress (copper-zinc superoxide dismutase [Cu/Zn SOD] and ferrochelatase [FC]), cellular metabolic condition (glucose-regulated protein [GRP 75] and total small heat shock proteins [sHsp]), protein metabolic condition (ubiquitin and heat shock protein 60 [Hsp 60], indicators of xenobiotic response (cytochrome P450-2 class [CYP 2], cytochrome P450-6 class [CYP 6], cnidarian glutathione-S-transferase [GST] and multi-drug resistance protein [MDR]). Details are provided in Fauth et al. (2006).

Corals were sampled with a 1.5cm punch, placed in opaque canisters underwater, blotted dry on deck, frozen on dry ice, and stored at -80° C. Frozen samples were ground to a powder, proteins extracted

in buffer, repeatedly centrifuged to generate a soluble protein (TSP) and assayed according to methods described in Downs (2005).

Station	Ridge position	Depth (m)	Reference Location	Treatment
HWO2	Middle	9	Hollywood	Ocean outfall
HWO3	Outer	16	Hollywood	Ocean outfall
PE2	Middle	8	Port Everglades	Inlet/ship harbor
PE3	Outer	15	Port Everglades	Inlet/ship harbor
HI2	Inner	9	Hillsboro Inlet	Ocean outfall + inlet
HI3	Outer	16	Hillsboro Inlet	Ocean outfall + inlet
FTL1	Middle	9	Fort Lauderdale	Control
FTL3	Outer	17	Fort Lauderdale	Control

Table 1. Sampling sites (see Banks et al 2007 for precise location)

Coral Colony Health

Health of coral colonies was assessed by quantifying the rate at which hole-punch lesions healed (Fisher et al. 2007). Five colonies at each site were sampled in January, 2005, and re-measured 8 mos later. Lesion area was calculated as an ellipse, and regeneration rate expressed as $mm^2\ d^{-1}$. We also estimated percent old and recent tissue loss using Atlantic and Gulf Reef Rapid Assessment (AGRRA) protocols (Kramer et al. 2005).

Ecosystem Assessment

Digital video 15-25 m transects were used to estimate percent cover. Video was converted to single-frame for pointcounting (15 pts./frame) to estimate percent projected cover of uncolonized (bare) substrate, stony corals and other functional groups: gorgonians, zooanthids, porifera, macroalgae, and the cyanobacteria *Lyngbya* (Dustan et al. 1999).

Statistical Analyses

Multivariate analysis of variance tested the null hypothesis that reef location (inshore versus offshore), and proximity to treated wastewater outfalls and shipping channels (diagnostic responses transformed as $\log_{10}(x + 1)$ had no effect on coral responses . Separate univariate tests interpreted significant MANOVA results and Tukey's Honestly Significant Difference separated univariate means. Hierarchical clustering revealed patterns in coral diversity, bottom cover and cellular-diagnostic responses. All analyses used JMP V. 4.0.4 (SAS Institute, Inc., Cary, NC, USA) at $\alpha = 0.05$.

Results

Community Composition

Sites clustered into two groups. Three inshore sites (FTL1, PE2 and HWO2) had the lowest substrate cover, highest *Lyngbya* , and least cover by sponges, stony corals and soft corals. All four offshore sites plus the inshore HI2 site clustered together because they had the highest percentages of bare substrate, the lowest cover of *Lyngbya*, and the most cover by sponges, stony corals and soft corals. (Fig. 1).

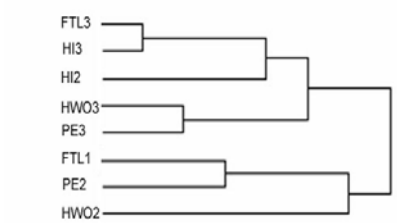

Fig 1. Sites clustered by community composition as percentage cover of functional groups described in text.

Cellular-Diagnostic Responses

Significant variation in the vector of ten cellular-diagnostic responses was explained by depth ($P < 0.001$), proximity to ocean outfalls ($P < 0.003$), depth x shipping channel ($P < 0.04$) and ocean outfall x shipping channel ($P < 0.02$). Eight parameters contributed most to the significant multivariate response: Hsp 60, Grp 75, ubiquitin, CYP 2, CYP 6, FC, MDR and GST. Accumulations of sHsp and Cu/Zn SOD did not vary with depth or proximity to potential land-based sources of pollution (Fig. 2).

Offshore corals, averaged across all sites, had significantly more Hsp 60 (protein metabolic condition) than did inshore corals ($P < 0.006$). Colonies from HI, a site near both a treated wastewater outfall and shipping channel, had less Hsp 60 than expected (significant interaction term: ANOVA $F_{1,29} = 10.57$, $P < 0.003$). Ubiquitin levels varied with the depth x shipping channel interaction ($P < 0.043$). Corals at inshore sites without a nearby shipping channel had twice as much ubiquitin as colonies at inshore sites with a shipping channel nearby. In contrast, corals at offshore sites had intermediate ubiquitin levels (Fig. 2)..

Grp 75 levels varied with the ocean outfall x shipping channel interaction ($P < 0.001$). Averaged across both depths, corals at HWO had more than three times as much Grp 75 as colonies at the biomonitoring and HI sites. FC levels varied significantly with the depth x ocean outfall interaction ($P < 0.03$). Corals at inshore sites near ocean outfalls had less FC than colonies at inshore sites without an ocean outfall and offshore sites with an ocean outfall nearby. Offshore colonies at sites without an ocean

outfall were indistinguishable statistically from these two groups (Fig. 2).

CYP 2 and GST levels were both higher offshore ($P < 0.02$). Averaged across depths, sites without shipping channels had CYP 6 levels 26% higher than sites with shipping channels ($P = 0.045$). Mean MDR levels varied with depth, ocean outfall, and the ocean outfall x shipping channel interaction (all $P < 0.034$). On average, mean MDR levels were 26% lower inshore compared to offshore sites, and 30% lower near ocean outfalls compared to those without them. In addition, mean MDR levels were significantly higher at the FTL biomonitoring sites than at the two HWO sites. Mean MDR levels at the paired PE and HI sites were statistically indistinguishable from both of these groups (Fig. 2).

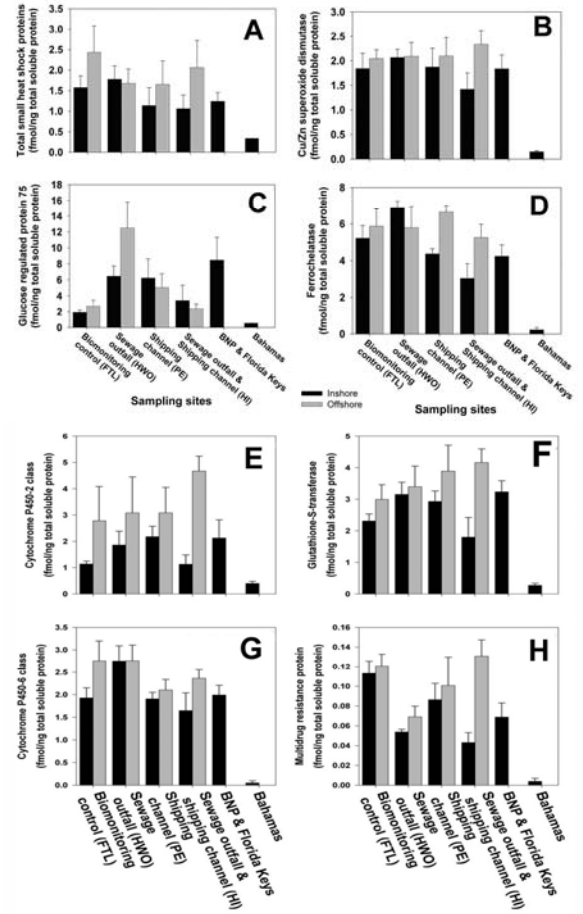

Fig. 2 Mean (± SE) accumulation levels for small heat shock proteins (A), Cu/Zn superoxide dismutase (B), glucose regulated protein 75 (C), ferrochelatase (D), Cytochrome P450-2 (E), glutathione-S-transferase (F), Cytochrome P450-6 class (G),multidrug resistance protein (H).

Coral Lesion Healing

Lesion regeneration rate varied significantly with depth and the sewage outfall x shipping channel interaction (Table 5). Coral colonies at all inshore sites except PE had lesion regeneration rates

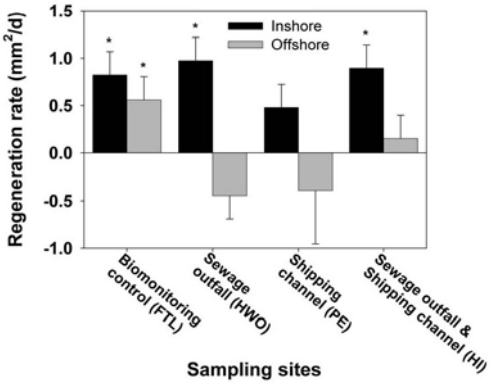

Fig 3. Regeneration rates (mm^2/d) of mustard hill corals (*Porites astreoides*). Data are least squares means (± 1 SE) from analysis of covariance, with initial lesion size as the covariate. Asterisks signify regeneration rates that differed significantly from zero. N = 1-5 colonies at each location.

significantly greater than zero (Fig. 3). In contrast, among the offshore sites only colonies at the biomonitoring control site had regeneration rates significantly greater than zero. Rates at the three other offshore sites were indistinguishable statistically from zero; mean regeneration rates at the offshore sewage outfall (HWO3) and shipping channel (PE3) were negative (Fig. 3).

Correlated Responses

Developing prognostic indicators of coral condition requires linking parameters of molecular and cellular function with the fitness of individuals and ecosystem structure and function (Depledge et al. 1993, Moore 2001, Fauth et al. 2003, Downs et al. 2005). In *Porites astreoides* sampled off Broward County, percent tissue loss regressed positively on GRP 75 accumulation (regression equation: Log_{10}(% mortality + 1) = 0.48 + 0.042[GRP 75 + 1]; hereinafter, concentrations in fmol/ng total soluble protein; $P < 0.008$, $R^2 = 0.20$), which is essential for cell proliferation. Backward stepwise selection identified a log-log model with Grp 75, ubiquitin, CYP 2 and CYP 6 as predictors of percent tissue loss. Coral colonies with low ubiquitin levels and high levels of Grp 75, CYP 2, and especially CYP 6 lost the most tissue (regression equation: Log_{10}(% mortality + 1) = $1.32 + 0.042\log_{10}$[GRP 75 + 1] - $0.84\log_{10}$[ubiquitin + 1] + $064\log_{10}$[CYP 2 + 1] + $2.15\log_{10}$[CYP 6 + 1]; $P < 0.005$, $R^2 = 0.40$). Similarly, levels of ubiquitin, CYP 2 and CYP 6 were significant predictors of lesion regeneration rate (regression equation: Log_{10}(% mortality + 1) = $-0.091 + 1.03\log_{10}$[ubiquitin + 1] – $1.32\log_{10}$[CYP 2 + 1] - $3.14\log_{10}$[CYP 6 + 1]; $P < 0.005$, $R^2 = 0.40$). Coral colonies with high ubiquitin levels and low levels of CYP 2 and CYP 6 possessed highest regeneration rates (Figure 4).

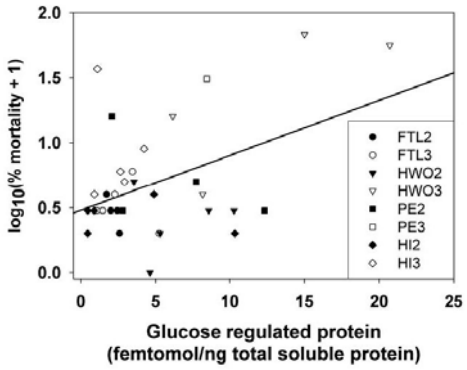

Fig 4. Log-linear regression of coral tissue mortality (%) as a function of glucose regulated protein (mortalin). Regression equation: Log 10 (mortality +1) = 0.48 + 0.042 Grp 75 (femtomoles/ng soluable protein). $F_{1,32}$= 8.10, P<0.008, R^2 = 0.20.

Comparisons with other Geographical Locations

Accumulations of every cellular-diagnostic parameter at the eight Broward County sites were indistinguishable from *P. astreoides* sampled the same month in Biscayne National Park and the Florida Keys. However, all biomarkers at every U.S. site were higher than those of *P. astreoides* from offshore Little Exuma Island, the Bahamas (two colonies, located approx. 23°30 N, 75°23W). The only exception was Hsp 60, which was undetectable in Bahamian corals and not differ from zero in inshore HI colonies. Cluster analysis (see Fauth et al. 2006) confirmed these analyses and the two Hollywood outfall sites (HWO2 and HWO3) clustered with FKNMS (Florida Keys National Marine Sanctuary) corals; the remaining inshore and offshore sites formed natural groups.

Discussion

Coral responses at the cellular, individual, and community levels provide a comprehensive assessment of the potential effects of land-based sources of pollution. Considered together, they suggest that corals off the southeastern Florida coast are exposed to poor-quality water, which reduces the ability of colonies to repair small lesions such as those created by our biopsy punches or by herbivores such as parrotfishes. In turn, this may reduce the ability of corals to colonize, grow and reproduce, thereby contributing to the low coral cover typical of this area (Banks et al. 2007). A study conducted further south in the Florida Reef Tract (Fisher et al. 2007) drew similar conclusions, which suggests this pattern may be widespread and common.

In our study, cellular diagnostic parameters indicative of xenobiotic stress were elevated at all our stations and in Biscayne National Park and Florida Keys National Marine Sanctuary, compared to corals in the Bahamas. This broad pattern illustrates the need to include a control distant from major sources of anthropogenic stress. *Porites astreoides* from the Bahamas had very low levels of all cellular-diagnostic parameters, and total sHsp was below detection limits there. High levels of cnidarian sHsp at all eight Broward County stations indicate they were responding to an oxidative stress (Downs et al. 2006).

The two stations near the Hollywood treated wastewater outfall and in the FKNMS possessed high levels of GRP 75, or mortalin. This enzyme is induced by glucose deprivation and involved cellular senescence and transformation. Elevated GRP 75 levels were associated with increased amounts of coral tissue loss. Colonies at these sites also had high levels of ubiquitin, which tags damaged proteins for degradation. Combined, these results suggest treated wastewater alters coral nutrition by generating higher than normal protein turnover rates , which inhibits coral growth and recruitment and results in decreased coral cover (Pastorak and Bilyard 1985, LaPointe et al. 2004).

Elevated levels of Hsp 60, CYP 2 and MDR at offshore sites FTL3, PE3 and HI3 are consistent with oxidative damage caused by exposure to xenobiotics. Cytochrome P450-2 class is induced by electrophilic carcinogens, drugs, and other environmental pollutants, which it oxidizes in a cellular suicide reaction. Glutathione-S-transferase conjugates the oxidized xenobiotic to glutathione, which is then pumped from the cell by multidrug resistance protein thus lowering the intracellular concentration of toxic compounds below their level of toxicity (Bard 2000). Increases in MDR usually occur only in response to an organic xenobiotic (Bard 2000; Sauna et al. 2001). The antibody used in this study binds both the cnidarian and dinoflagellate isoforms, hence results are a composite of MDR expression in both. Together, these results are consistent with offshore corals at the FTL3 biomonitoring site and off Port Everglades and Hillsborough Inlet reacting to exposure to anthropogenic contaminants. Regeneration of sampling lesions was negatively correlated with elevated levels of CYP 2, which suggests that mounting xenobiotic defenses had a metabolic cost: impaired ability to repair tissue damage.

Coral colonies at three inshore stations (FTL1, PE2 and HI2) and Biscayne National Park were characterized by cellular-diagnostic responses that tended to be lower than at the other Broward County sites. Corals at these three inshore stations also had moderate to high regeneration rates and little tissue loss, which is consistent with the defense trade-off hypothesis. While seemingly contrary to conventional wisdom, nearshore patch reefs in the

Florida Keys lost less live coral cover than more offshore reef communities (Porter et al. 2001). On such inshore reefs, coral colonies appear to be in better condition than conspecifics at offshore reefs, in part because inshore colonies accumulate defensive compounds more rapidly and return to homeostasis quickly once stressors recede (e.g., Downs et al. 1999; Fauth 2004; Downs et al. 2005). Inshore habitats are intrinsically more variable and much of the difference between inshore and offshore corals may have an ecotypic basis.

Total tissue loss was greatest near the City of Hollywood's treated wastewater outfall and moderately high at the offshore Port Everglades and Hillsborough Inlet stations. These three stations also had lesion regeneration rates indistinguishable from zero, and in two cases (HWO3 and PE3) tended to be negative; lesions grew larger instead of healing. Inability to regenerate small lesions indicates that conditions were poor for coral growth and reproduction at these sites between January and August, 2005.

Greatly elevated levels of cellular-diagnostic parameters and low coral cover at all sites we sampled off Broward County are cause for concern, especially because regeneration rates were indistinguishable from zero at four of eight stations. Our study establishes a line of evidence suggesting that land-based sources of pollution negatively affected the status and trends of these coral reef communities, which therefore should receive greater protection from potential damage caused by these stressors.

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Area-Calibrated Automation of Coral Classification for Near And Subsurface Reef Videos

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Abstract. Groundwork for an area-calibrated computer-automated system for benthic classification through underwater video is presented. Data acquisition through an underwater video camera is fast, less expensive and processing can be done in one day. Two video acquisition schemes were considered: (1) near-reef videos, where height of 30 cm from the reef surface is maintained, and (2) subsurface video of a reef where the camera is fixed 0.2-0.5m below the surface. Rapid classification is implemented via downsampling a reef image into blocks. Benthic components are classified into living and nonliving categories. For near-reef videos, an overall success rate of 79% is achieved even for corals occurring in various morphologies. Color and texture features derived from video stills were used as inputs to the classifier system. For subsurface reef video, an overall recognition rate of 60 – 70% was achieved. A more accurate percent cover is obtained via an area calibration model developed. This model is based on camera optics and removes the need for an underwater reference object for area correction. The development of an automated rapid reef classification system is most promising for reef studies that need fast and frequent data acquisition of percent cover of living and nonliving components.

Key words: coral classification, reef monitoring, automation, computer vision, pattern recognition

Introduction

The general approach to coral reef monitoring is determining population of benthic organisms or components in a reef such as living coral, dead coral, algae and sand, rock or rubble (Kenchington and Hudson 1984). Data on percent cover of different benthic components in a reef also helps in assessing damages or degradation on the reef incurred by trawling, storm or anthropogenic factors. A framework for an automated coral reef classification system from underwater video captured from near-reef and subsurface locations is presented. The goal is to automatically identify benthic components and to output an accurate percent cover with appropriate area calibration. An automated classification system is applicable for fast and frequent monitoring of reef organisms for temporal observations which can be correlated at once with specific occurrences, e.g. storm, dynamite fishing, etc. Investigation towards reef community studies can also be done using the system.

The science of determining benthic population data depends on the scale needed for assessment. For reef areas needing a resolution of at least 25 m^2, the commonly-used methods for monitoring are multi-spectral satellite imagery and aerial remote sensing (Mumby et al. 2004). Results from this method however need on-site validation. A limitation as well is the monetary cost of one remotely-sensed image. The method introduced in this study is a rapid, low-cost technique for on-site benthic cover estimation.

To estimate benthos distribution on site, several protocols have been put in practice such as manta tow, and Line-Intercept Transect (LIT) (Kenchington and Hudson 1984) which is an application of SCUBA. Recent monitoring methods such as Video Point Sampling (Uychiaco et al. 1992; Carleton and Done 1995) employ image and video capture in LIT to reduce diving time by allowing the counting to be done in the lab through playback. At playback, random points are generated on the screen and distribution is estimated by identifying and counting the objects directly underneath the points. Software such as *Coral Point Count with Excel* (Kohler and Gill 2006) has been developed for determining coral and substrate coverage using advanced methods in image processing. However, the method uses random point count sampling, which still requires user intervention and expertise in identifying the benthic components underneath overlaid points on the video frames. The aforementioned methods are quite taxing and take weeks to complete data collection for a reef span of only a kilometer. The tediousness of analysis and the long duration of time for acquisition and processing are not practical for marine management studies that require rapid observation of the effect of ecological disturbances on a reef that can occur in a matter of days.

The first published research on benthic component classification using computer vision techniques were applied to conventional LIT video and subsurface video (Marcos et al. 2005; Marcos et al. 2007). These papers introduced good classification for near-reef and subsurface video. In this study, area calibration is applied to benthic classification for underwater video using simple mathematical derivations from camera optics. This provides a more accurate benthic percent cover that accounts for changing camera-reef distances especially for subsurface videos. Area calibration removes the need for submerged reference objects for area estimation. The applicability of the automated system on new reef areas with a comparison on the results from a five-point sampling method is also presented in this paper.

Color and texture are utilized as cues to assign unique numerical identifiers for benthic categories. Color features are reliable indicators because it has minimum if not negligible variability across image scales and illumination. Also, there is good separability in color feature space for living and dead corals which are predominantly chromatic and achromatic, respectively. Texture features are utilized to distinguish living benthos, often occurring in regular and smooth textures, from sand and rubble substrates that generally have irregular textures. The combination of color and texture features can distinguish dead corals and dead coral with algae categories.

Material and Methods

Near-reef videos are acquired through LIT method via video capture done at approximately 30 cm from the reef surface. Underwater videos of coral reef transects from Talibon, Bohol (FISH Project, Marine Science Institute, UP Diliman) are utilized. Classification categories are living (live coral and algae) and nonliving components (dead coral, sand and rubble). 625 sub-images are employed for the training and the test set (living: 240; nonliving: 385). Selection of these sets is based on clarity and vividness of contrast and color. Alive corals are generally colorful and regularly textured while nonliving components are achromatic. Ground truth is provided by the Marine Science Institute of UP Diliman.

The subsurface reef videos are acquired in Ngedarrak reef, Republic of Palau. A Sony Model 2020IR bullet-type underwater submersible camera and an echo sounder are lowered on a motor-powered boat about 0.2-0.5 m from the water level. Boat speed is maintained at around 2 knots. The echo sounder was attached beside the video camera to simultaneously estimate the depth. Video acquisition was done on April 14, 2006.

Raw image sizes of 480×720 pixels were reduced to half (240×360 pixels) for fast computation. Percent cover from reef video is obtained by "downsampling" (Marcos et al. 2007) the video frame into small blocks (30×30 pixels), and each block is classified whether it is living or nonliving. Doing this for all blocks in a video of a reef area vis a vis per pixel classification provides a fast measure of living and nonliving benthos distribution. Prior analysis of the effect of varying block sizes and video frame interval sampling have found that recognition rates do not differ by more than ± 5-6%.

Fig. 1 illustrates video acquisition of the camera throughout the reef topology. Because the reef has variable height, the field of view varies with changing camera-reef distances (e.g. regions 1 and 2 in the figure). When counting the distribution of a benthos type, it will have more block counts for the shallower depth (region 1) than in greater depths (region 2) for a fixed actual benthos size/area. It is then more appropriate to estimate physical area distribution rather than using point-intercept method in reporting benthic distribution especially for subsurface video. For the system to obtain accurate and reliable percent count of any benthos, area calibration is needed.

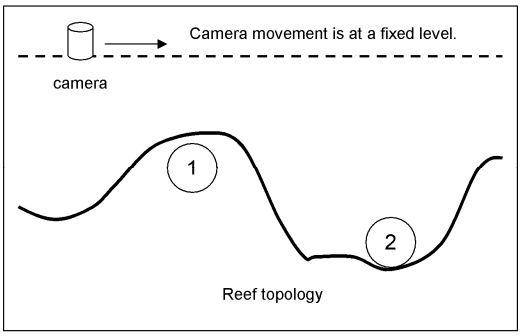


Figure 1: Illustration of video acquisition at different heights of the reef topology. Percent cover counting should be corrected for varying field of view due to changing distance between camera and reef.

For pinhole imaging as shown in Fig. 2, the field of view angle of an imaging system varies with object distance by the following equation:

$$\alpha = 2\tan^{-1}\left(\frac{I_V}{2x_I}\right) \quad (1)$$

where x_I is the lens to imager distance and I_V is the vertical measurement of the camera's imager. From the camera optics of Figure 2.6 combined with the thin lens equation: $1/x_O + 1/x_I = 1/f$, where x_O is the lens to object distance and f is the focal length, the actual vertical and horizontal field of view equation for a CCTV camera is derived as:

$$H = I_H\left(\frac{x_O}{f}\right); \qquad V = I_V\left(\frac{x_O}{f}\right) \quad (2)$$

where I_H and I_V are the horizontal and vertical sizes of the CCD imager of the camera, respectively. For underwater video capture, imaging variation due to the index of refraction of sea water is accounted through Snell's law where seawater decreases the field of view by 33%. Hence by multiplying I_H, I_V, $1/f^2$ and 0.33 a calibration factor C can be obtained for any submersible CCTV camera:

$$FOV_{horiz} \times FOV_{vert} = 0.33 \frac{I_H I_v}{f^2} x_0^{\,2} = C x_0^{\,2} \quad (3)$$

A calibration factor of $C = 0.44$ was found for the camera setup used in this study. This means that area calibration can be done when there is numerical data on camera specifications and camera-reef distance (obtained through echo sounder). Thus a submerged reference object is no longer required.

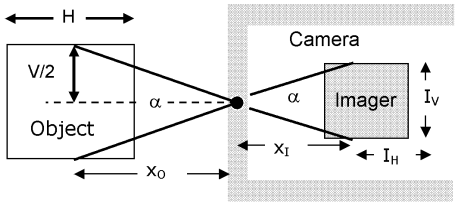


Figure 2: Basic optics of a CCTV camera

Color and Texture Features

The color features in (Marcos et al. 2005), the normalized *r-g* color space, are used which was found to be the best color feature among several color spaces tested for benthic classification. The color feature is computed from the RGB pixel values of the raw image frames from video.

A texture feature is a numerical description of intensity patterns (or patterns of pixels or group of pixels) in an image area. The rotation-invariant Local Binary Pattern histogram (Ojala et al. 2002) is utilized because it was proven to perform well on rotated, tilted and illumination-varied three-dimensional textures (Maenpaa et al. 2004). It was also shown to be a suitable texture descriptor for benthic components (Marcos, et al., 2005).

Classifier

Linear Discriminant Analysis (LDA) which is based on Bayesian theory is used as classifier. LDA is simple to calculate from data and is reasonably robust, i.e. the results are good even when the classes do not have normal distributions (Kuncheva 2004). Using the classification criterion to minimize total error of classification (TEC), the classifier will attempt to make the proportion of object that it misclassifies as small as possible. LDA employs Bayes Rule to assign an object to the group with highest conditional probability. All probabilities are assumed to have a multivariate normal distribution and all groups have the same covariance matrix C. An x_k is assigned to group i with the maximum value of

$$f_i = \mu_i C^{-1} x_k^T - \tfrac{1}{2} \mu_i C^{-1} \mu_i^T + \ln(p_i) \quad (5)$$

where μ_i is the mean of group i and p_i is equal to total sample of each group divided by the total samples.

Ground Truth

Expert-analyzed ground truth data was used to verify the output benthic cover of the system. To facilitate a fast point-by-point analysis (Fig. 3) a Matlab-based Graphical User Interface coral reef ground truth system (GUI-Coral Truth) was developed. The GUI-Coral Truth allows all sections of an image to be classified into five categories: coral, dead coral, dead coral with algae, sand and rubble for depths of 1 to 8 meters. Due to poor visibility at depths of more than 6 meters, ground truth error from visual inspection of the videos is expected to be large.

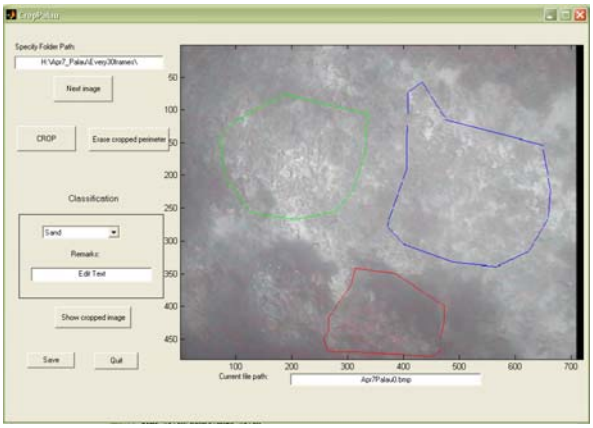


Figure 3: Graphical User Interface (GUI) developed for ground truth establishment

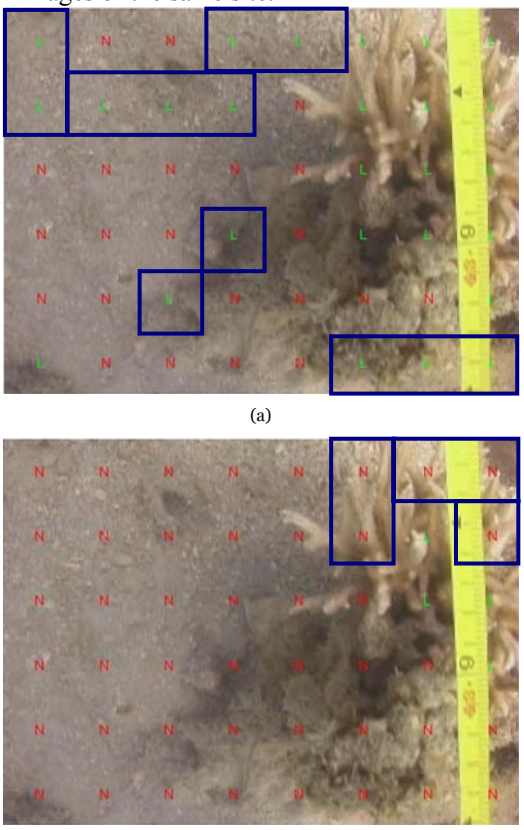
Results and Discussion

Near-reef Video

Table 1 shows the result of classification for the Bohol reef images where 79% recognition rate is obtained. This result verifies the potential of the classification system for identifying coral reef components from video of different reef areas.

Benthic cover result from a training set obtained from the same test site (Bohol reef) is compared with the result from a training set of a foreign/different site (Table 1). A non-zero trace with zero off-diagonal elements indicates a perfect (100%) recognition rate in a confusion matrix. The difference in percent cover count for foreign vs. same site training set reveals site-specificity in choosing the training set for classification. This is expected mainly because of different water quality conditions for both sites, which affects the signal-to-noise ratio of images acquired. Fig. 4 show visual comparison of this result Most

regions in the image are misclassified when a foreign training set is used. It is recommended then that training images for a specific reef area be taken from reef images of the same site.



(a)

(b)

Figure 4: Labeled Bohol reef images from percent cover count obtained through our classification system. Bold perimeter boxes designate regions of misclassification. (a) Result from using AIMS training set; (b) Result from using training set from the site itself.

Table 1: Confusion matrix for classifying Bohol reef images using combined color and texture. Classifier used is LDA.

Category	Living	Nonliving	Recognition Rate
Living	175	68	72%
Nonliving	65	317	83%
Overall Recognition Rate			79%

Subsurface Video

The subsurface benthic classifier achieved a high success rate in identifying living components especially at shallower depths, reaching a high 85% recognition rate at 3-meter depths. 75% recognition rate was obtained for nonliving components. Incorrect classification is attributed to occurrences of non-achromatic rubble images due to shadows cast by their structures and/or algae growing on them. A decline in success rate was observed with increasing camera-reef distances, as expected with the decrease in visibility . The automation was accomplished in approximately two hours using Matlab software and a computer with a Pentium 4 processor (1.5 GHz) and 1 GB memory.

Percent Cover with Area Calibration

To validate the area calibration in eq. 3, real-world area of field of view obtained from image measurements was plotted against various camera-object (resolution target) distances as shown in Figure 5. A quadratic fit of $y = 0.442x_O^2 + 0.512x_O$ was computed, where y is the area of field of view and x_O is the object distance from the camera. This is in good agreement with the derived calibration factor (Eq. 3) of $0.44x_O^2$. The second term in the quadratic fit is attributed to nonlinear factors inherent to the camera optics and the water column (refractive index of sea water may not be constant or equal to 1.33). It is also ascribed to skewing of the camera during video capture.

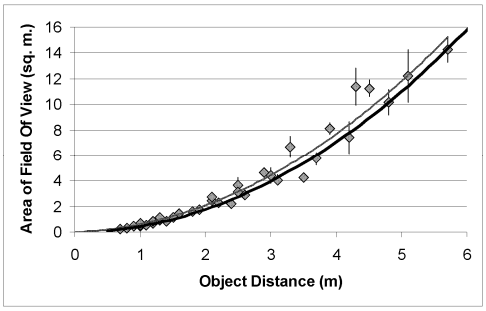


Figure 5: Validation of the area calibration factor. Data points represent actual field of view area measured directly from the images and processed via equation 12. The light trend line denotes a quadratic fit of $y = 0.442x_O^2 + 0.512x_O$ ($R^2 = 0.96$), where y is the area of field of view and x_O is the object distance from the camera. The field of view calibration factor we derived in equation 3 ($y = 0.44x_O^2$) is represented by the bold line.

The actual spatial area cover of living and nonliving components of subsurface reef video captured from the Ngedarrak Reef, Palau was computed after area calibration. Depths greater than 6 meters produce video frames that lack visual clarity and thus were excluded because of a large uncertainty in ground truth establishment through video inspection. Cumulative area cover of living and nonliving components was computed at increments of 1 meter camera-reef distance (Table 2). This will determine the maximum depth to acquire subsurface video where accuracy of percent area cover may fall below acceptable values. It is emphasized though that acceptability of any experimental result is dependent on the end user. From Table 2, it can be surmised that for a user requiring a spatial cover accuracy of more than 70%, the classification system can be applied to videos acquired up to depths of 2 meters. It is emphasized that identification of coral reefs only at depths of 2 meters is already immensely useful for marine scientists especially when analyzing results of

environmental catastrophes such as typhoons which affects shallow reefs in general.

Table 2: Comparison of cumulative derived area of living and nonliving components from classification system and from ground truth. The success rate based from ground truth area are indicated.

Camera -reef distance	Area derived from classification (m2)		Area derived from ground truth (m2)		% Success Rate
	Living	Nonliving	Living	Nonliving	
1 to 2 m	0.73	0.38	0.93	0.18	75.70
1 to 3 m	6.65	3.35	6.54	3.46	56.20
1 to 4 m	14.52	7.83	12.60	9.75	46.90
1 to 5 m	22.18	15.13	19.17	18.15	46.10
1 to 6 m	26.19	17.30	24.65	18.85	48.00

Comparison of Method with Video Point Sampling

The system developed in this study for subsurface video analysis of reef video is compared with video point sampling using five points as markers. The point sampling classification was done by Mr. Victor Ticzon of the Marine Science Institute, UP Diliman. Subsurface video was sampled from 9 stations along the Lingayen gulf, Pangasinan.

Comparison of computed hard coral cover from both methods are illustrated in Fig. 6. For the automated classification, training images were obtained from the site itself. Nine of 11 stations analyzed by both methods have less than 10% difference in percent cover estimates of live coral cover. Small difference in these results reveals that both methods can gain similar or comparable results. It is emphasized though that frames that lack visual clarity are removed from the analysis in both methods. Although fixed point sampling is the standard method used by most marine scientists in determining percent cover, the classification system introduced in this paper exploits all information from the image, i.e. every region in the image is considered.

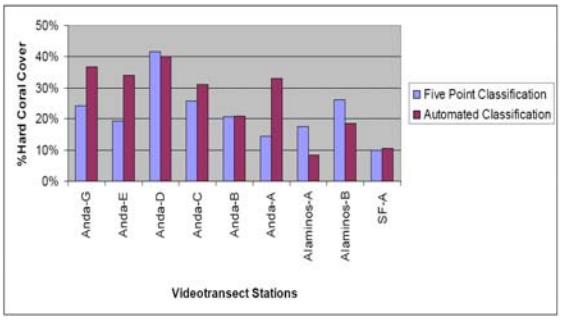


Figure 6: Bar graph of the percent cover of hard corals in 9 stations of the Lingayen gulf as assessed by five point classification and the automated classification developed.

This study on classification for near and subsurface reef videos provides groundwork for developing rapid automated systems for benthic classification and mapping using a simple reef transect video. Area calibration is introduced since point-intercept method is inadequate in reporting percent cover due to variability in camera-reef distances. This calibration model also eliminates the need for submerged reference objects. Although this study, which is the first to explore automation of subsurface benthic images, have obtained a fair accuracy rate in classifying living benthos, it is highly likely that improved image quality from better camera optics and electronics can yield higher recognition rates even at greater depths.

The applicability of the system's current performance would depend on the field study objectives and the needed accuracy. If rapid assessment of coral cover is desired especially in reef areas affected by typhoon and oil spill, then the system would suffice as long as video is taken at 2 meter depths. Although video point sampling protocol is the current standard in determining benthic cover because of its high recognition accuracy, this process is quite tedious and needs expert manning. Nonetheless, our system can be used to supplement results from multi-spectral imagery, and even video point sampling.

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Large scale coral mortality in Barbados: a delayed response to the 2005 bleaching episode

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Abstract. Corals in Barbados suffered widespread bleaching during the high temperature event of 2005. Six reefs were monitored for one year (October 2005 – November 2006) to determine mortality impacts and rate of recovery from this bleaching event. Five 1 x 20 m band transects and five 20 m line transects were quantitatively surveyed at each site, every four months. Bleaching prevalence dropped from a mean of 71% of colonies in October 2005 to 38% in February, to 17% in June, before rising again to 25% in November 2006. Coral mortality remained low for five months (means: 3.8% colony surface dead; or 4.8% dead cover), but rose sharply after 10 months (means: 18.7% colony surface; or 25.9% cover), eventually declining after 15 months to near ambient levels (means: 2.0% colony surface; or 6.1% cover). Like other eastern Caribbean islands, recovery from bleached condition was slow and overall mortality impact was high on both deep and shallow reefs. In contrast were the delayed onset of mortality and low incidence of coral disease. High losses in live coral cover have significant economic implications for the island which derives a major proportion of its GDP from tourism, and relies heavily on healthy reefs for coastal protection.

Key words: coral bleaching, mortality, Barbados.

Introduction

Coral reefs across the Caribbean suffered extensive bleaching as a result of the record high temperature event of 2005 (Donner et al. 2007; Souter and Wilkinson 2008). In particular, the eastern Caribbean Lesser Antilles experienced unprecedented heating stress, and high levels of coral mortality were reported for several islands by early 2006 (Miller and Muller 2006; Bouchon et al. 2008; Morgan et al. 2008; Woody et al. 2008).

In Barbados, bleaching impact was very high, but the initial mortality response was low compared with early reports from other eastern Caribbean islands (Oxenford et al. 2008). Here we examine the cumulative mortality impact of the bleaching episode on Barbados reefs over the ensuing year.

Methods

Quantitative surveys were conducted at three shallow (<10 m) and three deep (>15 m) reef habitats along the west and southwest coasts of Barbados (see Oxenford et al. 2008 for reef study site locations and descriptions) every four months from September/October 2005 to November 2006. At each reef site, five haphazardly placed 1 x 20 m belt transects and five rapid assessment 20 m line intercept transects were surveyed.

Extent of bleaching was assessed along the belt transects by recording the number and species of all coral colonies and the number suffering partial or full bleaching. This was presented as transect mean percent colonies bleached for each reef site.

Coral mortality was assessed using two independent indices: 1) visually estimated percent of each colony surface recently dead (recorded for every colony encountered in the belt transects by species), and 2) percent of benthic coral cover recently dead (measured directly along the entire length of the line intercept transects without regard to species identification). Recently dead coral was identified by the presence of newly exposed white skeleton with or without a fine layer of green turf algae (see Lang and Marks 2006). This 'state' was observed to last only a matter of weeks in tagged corals before reverting to 'old' mortality (Oxenford, unpubl. data). Therefore, cumulative mortality (calculated as the sum of recent mortality recorded each survey) was used to represent the full mortality impact of the bleaching event. Mortality data were presented as transect means per site for both indices.

Species complexes *Montastrea* (*M. annularis, M. faveolata, M. franksi*) and *Agaricia* (*A. agaricites, A. humilis, A. lamarcki, A. grahamae*) whose individual species could not be reliably identified by all observers, were treated as single species (*Monastrea* spp. complex and *Agaricia* spp. complex respectively) in the analyses.

Benthic water temperatures were recorded *in situ* at

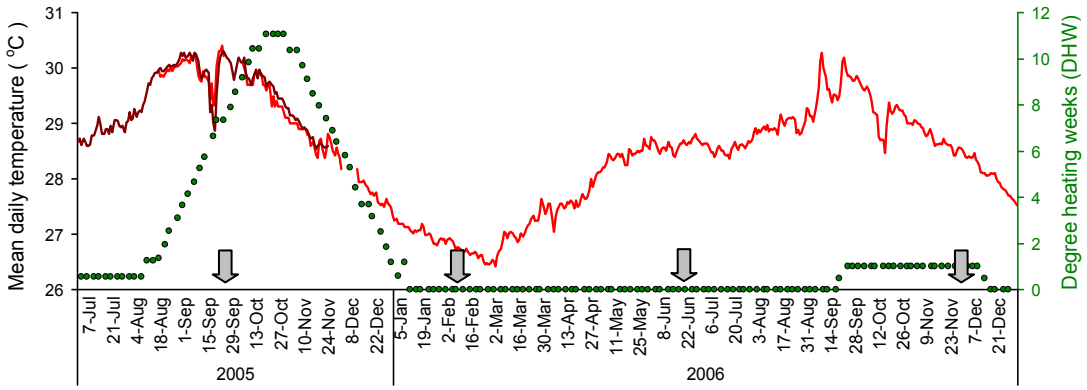

Figure 1. Benthic water temperatures recorded at two reef sites in Barbados, shown together with NOAA/NESDIS computed degree heating week data for the island. Dates of coral surveys are indicated by filled arrows.

deep and shallow sites every 4 h using HOBO® Water Temp Pro data loggers fixed in the reef. Computed mesoscale (50 km grid) degree heating week (DHW) data (Liu et al. 2006) were accessed from the archived NOAA/NESDIS Coral Reef Watch database at http://coralreefwatch.noaa.gov/satellite/hdf/index.html.

Results

The benthic water temperature and DHW time-series experienced by Barbados corals during late 2005 through 2006 are shown in Fig.1. In 2005 benthic water temperatures had exceeded 29.5 °C by the first week in August 2005 at both deep and shallow sites and persisted for almost three months. By comparison, in 2006 benthic water temperatures rose to 29.5 °C by the first week in September, but lasted for less than one month (Fig. 1). Heating stress was first apparent in Barbados in late May 2005, with values of 0.55 DHW persisting from May 24 - August 9. Heating stress rose sharply thereafter, reaching record highs of 11.05 DHW in the second half of October, and persisted through early January, 2006 (Fig. 1). The summer of 2006 was cooler with relatively low heating stress (1 DHW) occurring from late September to early December.

Bleaching was severe from late 2005 into 2006, affecting both shallow and deep reef habitats, virtually all hard coral taxa and the majority of colonies (see Oxenford et al. 2008), and persisted over the full year of study (Table 1). Bleaching prevalence gradually declined from a high of 70.6% of all colonies across all reefs in late September/early October 2005 to 17.1% in June 2006, 10 months after the initial onset of bleaching. Prevalence then rose again to 25.3% in November 2006 after summer warming (Table 1, Fig. 1).

Shallow reefs suffered significantly higher levels of bleaching than deeper reefs initially (Mann-Whitney: $U = 18$, $n = 30$, $P < 0.001$), showed no statistically significant differences in February or June ($U > 69$, $n = 30$, $P > 0.200$ in both cases), but by November the situation had reversed, with deep reefs suffering on average significantly more bleaching than shallow reefs ($U = 55$, $n = 30$, $P = 0.017$; Fig 2).

Table 1: Summary of bleaching prevalence of surveyed reefs shown as mean percent of all colonies that were fully or partially bleached along five 1x20 m belt transects at each reef site. SE in parentheses.

Reef site		Survey Dates							
		Oct-05		Feb-06		Jun-06		Nov-06	
Type	Name	15 Sep – 6 Oct		6 Feb – 9 Feb		20 Jun – 22 Jun		27 Nov – 7 Dec	
Deep (> 15 m)	Welcome Inn	59.4	(6.8)	43.1	(3.0)	18.8	(3.6)	36.9	(5.7)
	Atlantis	63.0	(3.8)	43.7	(6.7)	25.2	(6.9)	37.0	(5.7)
	Maycocks	59.1	(2.1)	32.6	(2.7)	16.9	(2.7)	20.7	(4.4)
Deep reefs mean		60.5	(2.5)	39.8	(2.8)	20.3	(2.7)	31.5	(3.5)
Shallow (< 10 m)	Coconut Court	86.0	(1.3)	19.9	(1.5)	7.3	(2.6)	8.8	(3.9)
	Batts Rock	73.8	(6.7)	46.0	(1.7)	21.5	(3.7)	25.2	(4.1)
	Bellairs	82.0	(3.1)	39.7	(8.7)	13.1	(5.6)	23.5	(9.0)
Shallow reefs mean		80.6	(2.7)	35.2	(4.1)	14.0	(2.7)	19.2	(3.8)
All reefs mean		70.6	(2.6)	37.5	(2.5)	17.1	(2.0)	25.3	(2.8)

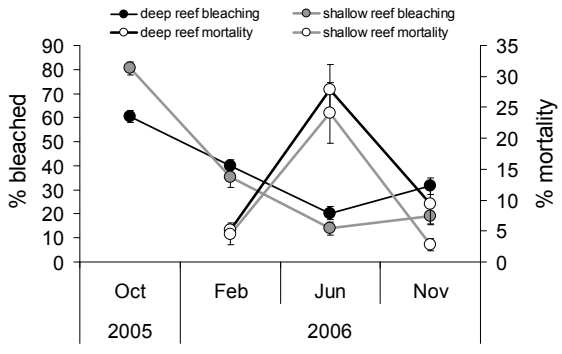

Figure 2. Mean bleaching and mortality responses of corals on deep and shallow reefs in Barbados to the 2005 hot water event. Bleaching is shown as mean % of coral colonies affected; mortality is shown as mean % of recently-dead benthic coral cover; bars show standard error.

The two coral mortality indices were highly correlated (R^2 = 0.904, n = 36), showing the same patterns among reef sites and the same trend over time (Fig. 3). However, the belt transect method, measuring whole coral colony surface, consistently estimated slightly lower mortality values than the rapid assessment, line intercept method measuring benthic coral cover (Fig. 3).

Bleaching-induced mortality, when first examined in February 2006, was relatively low across all reefs (mean % colony surface recently dead: 3.8% ±0.1 SE; mean % benthic coral cover recently dead: 4.8% ±1.0 SE), but rose sharply after 10 months (June 2006 – mean colony surface: 18.5% ±2.5 SE; mean cover: 25.9% ±3.2 SE), eventually declining after 15 months to near ambient levels (November 2006 - mean colony surface:2.0% ±0.4 SE; mean cover: 6.1% ±1.8 SE). Cumulative mortality impacts of the bleaching event are therefore estimated at approximately 24.3% loss in live coral colony surface or 36.8% loss of live coral benthic cover.

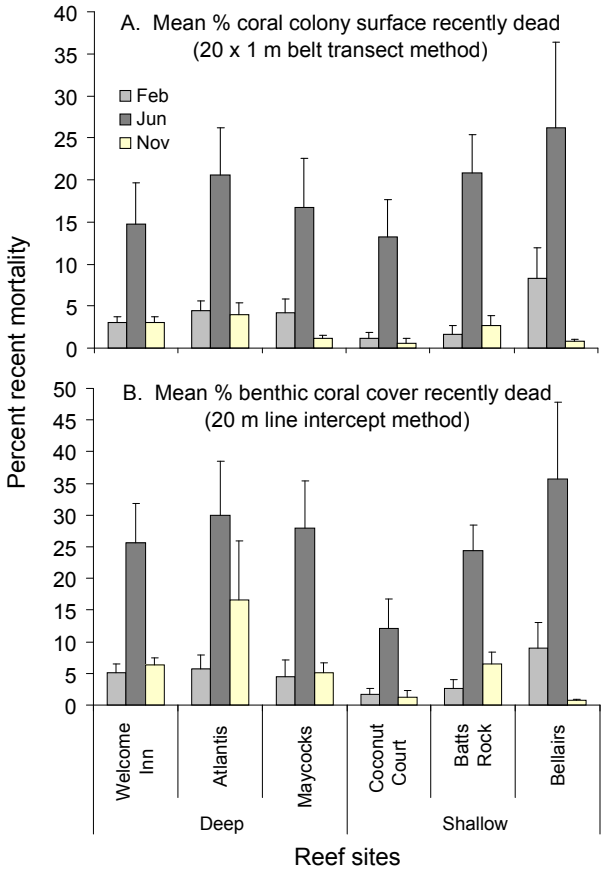

Figure 3: Mean percent loss of live coral at reef sites in Barbados following severe bleaching event of summer 2005, shown by two indices; A – mean estimated % of coral colony surface recently dead, and B – mean percent of benthic coral cover recently dead.

Similar to the trends in bleaching, mortality impacts did not differ significantly between shallow and deep reefs in February nor June 2006 (U > 86, P > 0.05 in both cases), but appeared greater on deep reefs than shallower reefs by November 2006, at least for the benthic coral cover index (Mann Whitney for coral cover: U = 54.5, n = 30, P = 0.015, Fig. 2; cf. colony surface: U = 70, n = 30, P = 0.078).

The severity of mortality impacts differed among coral species, but the trend was similar (Table 2). All species experienced the highest levels of mortality in June 2006, with the exception of *Favia fragum, Siderastrea radians* and *Millepora complanata* (Table 2). *F. fragum* and *S. radians* suffered very high levels of bleaching but low mortality throughout, whilst the milleporids suffered high levels of bleaching and very high early mortality, with *M. squarrosa* eventually disappearing completely from survey transects (Table 2).

Bleaching response was not always indicative of the longer term mortality response across all species. For example, among the most commonly observed species, *Agaricia agaricites*, *Montastrea annularis*, *Porites porites* and *Millepora alcicornis,* all suffered initial bleaching of more than 70% of all colonies and cumulative mortality in excess of 30% of colony surface (Fig. 4). In contrast, *Diploria strigosa* and *Favia fragum* experienced more than 70% bleaching but cumulative mortality was less than 12% for these two species, and *Colpophyllia natans* had very low level of bleaching (< 10%) but more than 30% mortality (Fig. 4).

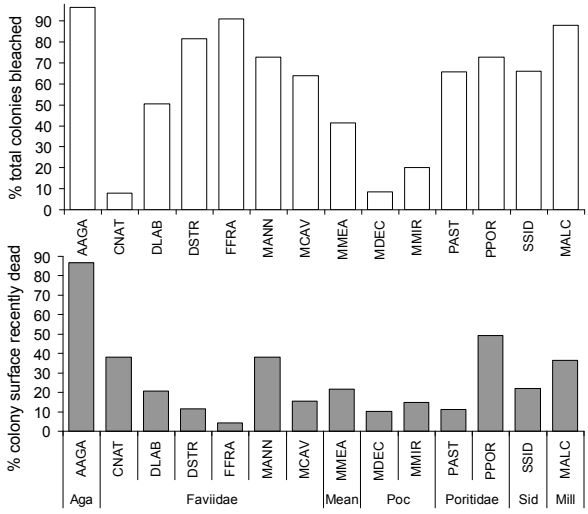

Figure 4. Initial bleaching and cumulative mortality impacts shown for the most common coral species (> 50 colonies per survey) over the period September 2005 to November 2006, grouped by family. Initial bleaching was recorded in September/October 2005. Cumulative mortality represents the sum of 'recently-dead' colony surface recorded in February, June and November 2006. Species codes are given in Table 2.

Table 2: Summary of bleaching and mortality census data for six reefs in Barbados shown for all colonies by species. Data were collected along five 1x20 m belt transects at each reef and are presented as percent of total colonies bleached by species, and percent of colony surface recently dead over all colonies by species.

Class	Family	Code	Species	Sept/Oct-05		Feb-06			Jun-06			Nov-06		
				n	% bleached	n	% bleached	% mortality	n	% bleached	% mortality	n	% bleached	% mortality
Anthozoa	Acroporidae	ACERV	*Acropora cervicornis*	0	-	0	-	-	1	0.00	0.00	0	-	-
	Agariciidae	AAGA	*Agaricia* spp. complex	1322	96.60	166	62.65	31.87	282	23.40	50.90	139	38.13	3.92
		LCUC	*Leptoseris cucullata*	1	100.00	3	33.33	0.00	2	0.00	2.50	10	20.00	0.00
	Astrooeniidae	SBOUR	*Stephanocoenia bournii*	0	-	0	-	-	6	33.33	0.00	0	-	-
		SINT	*Stephanocoenia intersepta*	18	38.89	35	25.71	1.00	41	36.59	5.34	46	41.30	0.87
	Caryophylliidae	EFAST	*Eusmilia fastigiata*	12	16.67	18	55.56	5.56	20	20.00	15.75	22	22.73	5.68
	Faviidae	CNAT	*Colpophyllia natans*	64	7.94	89	15.73	2.00	142	6.34	30.83	95	9.47	5.35
		DCLIV	*Diploria clivosa*	5	80.00	28	39.29	3.75	5	20.00	10.00	4	0.00	0.00
		DLAB	*Diploria labyrinthiformis*	75	50.67	85	31.76	2.16	106	14.15	17.53	113	22.12	1.14
		DSTR	*Diploria strigosa*	277	81.59	311	32.48	2.21	402	14.68	8.75	414	22.22	0.46
		FFRAG	*Favia fragum*	11	90.91	95	93.68	3.84	131	41.22	0.38	128	56.25	0.00
		MANN	*Montastrea* spp. complex	489	72.60	858	58.28	2.16	971	33.47	31.92	690	46.38	4.05
		MCAV	*Montastrea cavernosa*	238	63.87	386	26.17	1.15	431	10.44	12.65	577	18.89	1.79
	Meandrinidae	DCYL	*Dendrogyra cylindricus*	15	100.00	0	-	-	7	14.29	38.57	1	0.00	0.00
		DSTOK	*Dicocoenia stokesii*	10	30.00	10	10.00	0.50	10	10.00	11.00	15	26.67	3.33
		MMEAN	*Meandrina meandrites*	92	41.30	185	43.78	5.04	209	37.80	15.36	204	41.18	1.35
	Mussidae	IRIG	*Isophyllastrea rigida*	1	100.00	0	-	-	0	-	-	0	-	-
		MANG	*Mussa angulosa*	2	100.00	4	0.00	0.00	2	50.00	5.00	-	-	-
		MYCET	*Myceptophillia* spp.	2	0.00	3	33.33	0.00	4	0.00	0.00	5	0.00	0.00
		SCOLY	*Scolymia* sp.	1	0.00	0	-	-	0	-	-	0	-	-
	Pocilloporidae	MDEC	*Madracis decactis*	59	8.47	116	12.93	0.93	286	2.80	8.17	223	7.62	0.98
		MMIR	*Madracis mirabilis*	55	20.00	74	4.05	4.19	94	0.00	10.63	97	2.06	0.10
	Poritidae	PAST	*Porites astreoides*	1084	65.59	853	12.66	2.85	1553	5.54	7.51	1309	4.97	0.90
		PFUR	*Porites furcata*	0	-	0	-	-	0	-	-	17	0.00	0.59
		PPOR	*Porites porites*	250	72.80	138	26.81	16.74	268	2.24	31.48	162	3.70	1.00
	Siderastreidae	SRAD	*Siderastrea radians*	20	70.00	18	72.22	5.00	6	33.33	0.83	13	100.00	4.62
		SSID	*Siderastrea siderea*	318	66.04	238	76.47	2.49	333	42.04	15.80	337	68.25	3.71
Hydrozoa	Milleporidae	MALC	*Millepora alcicornis*	83	87.95	41	26.83	9.51	68	4.41	22.90	41	7.32	4.15
		MCOM	*Millepora complanata*	40	90.00	23	0.00	46.09	40	0.00	25.93	45	0.00	5.56
		MSQU	*Millepora squarrosa*	58	75.86	12	8.33	90.83	111	0.00	96.40	0	-	-
	Stylasteridae	SROS	*Stylaster roseus*	3	0.00	22	0.00	0.00	8	0.00	0.00	14	42.86	0.00

Discussion

Sea water temperatures and accumulated heating stress through the summer and fall of 2005 were unprecedented for the Caribbean region (Wilkinson and Souter 2008). Conditions in Barbados were no exception and resulted in the worst coral bleaching event ever recorded in this region (Oxenford et al. 2008). Similar claims have been made for other islands of the Lesser Antilles, where the hotspot developed and remained for many months (see NOAA/NESDIS archived database; Wilkinson and Souter 2008).

Although the initial mortality impact on corals was low in Barbados compared with many other locations (Oxenford et al 2008), prolonged bleaching resulted in delayed mortality with high losses of live coral, comparable to elsewhere in the Lesser Antilles (Bouchon et al 2008; Woody et al 2008).

The highest losses of live coral occurred 9 to 10 months after the initial onset of the bleaching event. Corals in Barbados did not appear to die as a result of any described syndrome. Incidence of coral disease was negligible throughout the surveys, in contrast with other reports from the Lesser Antilles (e.g. French West Indies and the US Virgin Islands) where infections were reported to be the primary cause of early bleaching induced coral mortality (Miller et al. 2006; Muller et al. 2008; Bouchon et al. 2008).

Differences in mortality estimates between the two indices used in this study and the delayed onset of mortality in Barbados compared with elsewhere serve to illustrate the importance of standardising methodologies and extending the time-frame over which reefs are surveyed when drawing conclusions about the impact of an event and/or making comparisons among geographically separated sites.

The difference among species in their bleaching and mortality response to the hot water event was marked, but relatively consistent among the few detailed reports from the region with *Agaricia agaricites*, *Colphyllia natans* and *Montastrea annularis* showing particularly high mortality (Woody et al. 2008). Differences in behavioural response such as the ability to increase the level of heterotrophic feeding (Grottoli et al. 2006), and in coral host and symbiont genetics (Berkelmans and van Oppen 2006) certainly contribute to the variation in resistance among species, but are not well understood.

This event marks the most extreme coral bleaching experienced by Barbados reefs on record and corroborates the prediction of more frequent and more severe mass coral bleaching events in the Caribbean with the current trend of global warming (e.g. McWilliams et al. 2005). The study also represents the first quantitative examination of mortality response to mass bleaching in Barbados and indicates the severe ecological impact with cumulative losses of 42.4% and 31.3% of live coral cover from deep and shallow reefs respectively. High losses in live coral cover have significant social and economic implications for Barbados, which relies on healthy reefs for marine-based tourism, coastal protection, and small scale reef-associated fisheries (NCSD 2004). The high coral mortality outcome certainly emphasises the vulnerability of tropical small island developing states (SIDS) like Barbados, with a high reliance on healthy marine ecosystems, to the progression of global warming. It further emphasises the urgency for improved national conservation and coral protection measures to mitigate local stressors and improve reef resilience, whilst continuing to lobby for international action to reduce climate change.

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Mapping reef structure and bathymetry in Belize using Cobra-Tac

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Abstract. The Cobra-Tac underwater navigation system (RJE Int, Irvine, CA) was evaluated for its mapping capabilities in shallow (< 20 m) hard- and soft-bottom reef habitats. This study was conducted on the lagoon reefs around Wee Wee Caye, Belize. The Cobra-Tac was created as an autonomous underwater diving navigation system that measures depth and altitude for hydrographic and position mapping. It computes its geographic position underwater using a fluxgate compass and a Doppler velocity log. Our objective was to assess the Cobra-Tac's effectiveness as a measuring tool for the rugosity of reef structure and for mapping bathymetry. We found that the accuracy, density, and breadth of data collection are dependent upon speed of travel and altitude above the bottom. Also, multiple passes over the desired area may be necessary to best define the underwater topography in detailed resolution. One advantage over boat-based systems is that Cobra-Tac is diver operated and can be taken into shallow and complex habitats. Provided that the limitations of the system are understood, the Cobra-Tac is a powerful tool for easily mapping the topography of small-scale and shallow marine systems. It produces intelligible and informative bathymetric maps within 30 minutes of data download that are then ready to be used to delineate transects for biotic surveys.

Key words: reef mapping, underwater navigation, Cobra-Tac, Doppler device

Introduction

Coral reefs are complex habitats that support diverse assemblages of biota. The structural rugosity of a reef can be a good predictor of the diversity and abundance of organisms that inhabit reefs (Grigg 1994). While the topographic complexity of reefs is expected to be influential in the abundance of most fishes (Luckhurst and Luckhurst 1978; Gratwicke and Speight 2005), attempts to correlate reef structure with fish density and species richness has met with mixed success. For example, Chapman and Kramer (1999) found a significant relationship between rugosity and fish density for only 10 out of 26 studied species. McCormick (1994) compared six different metrics of topography as indicators of fish abundance. He found a significant relationship in only 18 of the 50 species studied. The reason for this apparent disconnect between topography and fish abundance was due to the difficulty of measuring reef topography on a scale appropriate to the fish survey (McCormick 1994). The most common method for mapping topographic complexity is the chain method developed by Risk (1972). This method is typically applied over areas of 10 m or less. Although this scale may be adequate for smaller fishes, it may not capture the habitat at scales relevant to larger fishes (McCormick 1994; Bartholomew et al. 2008). Some researchers have recognized the scaling problem. When the spatial scale of habitat characterization was expanded, it resulted in better estimation of the abundances of roaming fishes (Friedlander and Parrish 1998; Abesamis et al. 2006; Mumby et al. 2007; Bartholomew et al. 2008). New quantitative methods of characterizing topographic complexity in shallow marine systems at larger scales (100's of meters) have not been fully developed.

Current reef mapping technologies are time intensive. Sidescan sonar provides habitat assessment over broad areas, but topographic relief is difficult to extract. Sidescan sonar does not offer consistent resolution, as the relative height of the towfish above the bottom can vary during operation. Diver surveys provide accurate biological inventories but the collection of directly comparable quantitative topographic information is difficult. Other methods exist (Risk 1972; Smith et al. 1975; Aronson et al. 1994; Mumby et al. 1997; Maeder et al. 2002; Fossa et al. 2005), but each of them, while effective in gathering some metrics, is limited by cost, time, quantity, or quality of data.

To address this need for improved bathymetric mapping technologies, we tested the Cobra-Tac

autonomous underwater navigation system (RJE International) for its potential to produce quick, high-resolution bathymetric maps of reef habitats. Cobra-Tac is a diver-operated acoustic Doppler profiler that utilizes tracking technology for underwater navigation.

Study Sites

The study sites were in Belize, Central America. Wee Wee Caye is a small island in the lagoon of the Mesoamerican Barrier Reef off Belize (16.76474288° N 88.142522° W). Southwater Caye is a small island abutting the barrier reef at 16.813567° N 88.080172° W. The surveys were performed in December of 2007.

Methods

Surveys with Cobra-Tac were conducted using a swimming or towed diver. Swimming divers navigated and operated the unit at various depths. A diver (or snorkeler given calm conditions and low speeds) was towed behind a boat (at speeds of 2-4 kt). The boat-towed setup allowed us to compare GPS readings directly with the Cobra-Tac navigational calculations.

The most effective data collection method involved towing or swimming the Cobra-Tac in a tight organized grid. In all cases, holding the unit absolutely horizontal so that the Doppler is aimed directly downwards is critical.

The unit requires the user to input initial GPS coordinates at the surface. The diver then swims the study site while the unit records the location and depth of four points (one for each beam) at one-second intervals throughout the survey. After the completion of the survey, post processing is necessary to create a three-dimensional map of the site. Maps are created with Surfer (Scientific Software Group) or the data can be exported to other software platforms.

Results

The Cobra-Tac and supplied software were readily capable of producing a three-dimensional image of a study site in about 30 minutes of post-processing. In addition, Matlab (Mathworks Inc.) was applied to the Cobra-Tac data to examine details and consistency of the original Cobra-Tac data set.

The Cobra-Tac's navigational accuracy was tested by performing swims along irregular tracks and then navigating back to the start point. Table 1 shows three trials where the start/end location was the same fixed structure. The results from these trials show an accumulated error between 8 and 31 m, which is well within the manufacture's specified 3-5% error. Accuracy could be improved by setting reference points along lengthy surveys for post-dive calibration.

Table 1: Distances traveled during each dive and the error from the starting point Cobra-Tac showed at the end of each dive

Approx. Distance Traveled (m)	Error in recognizing return to starting position (m)
500	8
1600	31
1600	27

The spur and groove reef mapped at Southwater Caye was created with a single pass of the Cobra-Tac operated by a snorkeler (Fig. 1). The spur and groove formations are clearly visible in the figure, but much fine-scale irregularity was lost due to the smoothing functions applied by the supplied software.

The channel immediately west of Wee Wee Caye was also mapped (Fig. 2). The map was created with 6 passes along the longer dimension of the channel, each the length of the channel in a single grid-formation tow. The island and the slopes of the shoals to the west and north are clearly visible in the image. The bathymetry of the channel-bottom, which otherwise is too deep to see from the surface, is also well defined.

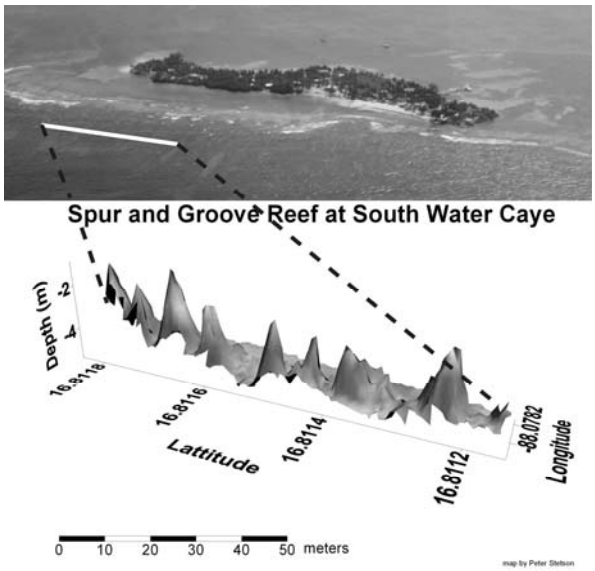

Figure 1: 3-D surface map (bottom) of single-pass transect over spur and groove reef at SouthWater Caye, Belize. The sampled area corresponds to the white line shown in the upper image.

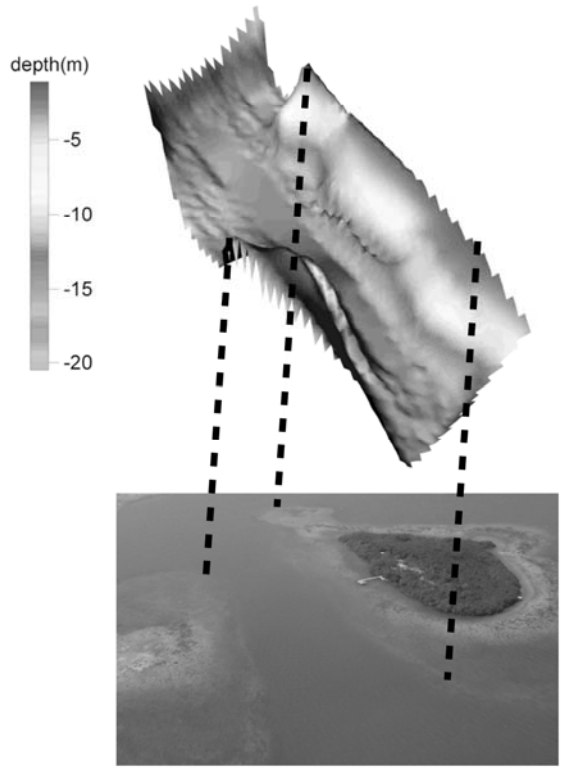

Figure 2: Surface map of channel west of Wee Wee Caye, Belize on top of aerial photograph of the caye. Dashed lines illustrate how the images line up.

To illustrate the necessity of adequate site coverage, the channel data set (Fig. 2) was reanalyzed using subsets of the total number of passes. Revised surfaces were created from these selected passes over the channel (Fig. 3). Too few passes resulted in inaccurate topographic detail.

The following formula was developed to determine the best mapping strategy for a given area in terms of how many passes (or tracks) are needed to yield optimum bottom coverage. The minimum number of passes, ***np***, over a study site is equal to the width of the site, ***w***, divided by the breadth of the beam angles. The breadth of the beam angles is equal to 2 times the height of the unit above the bottom, ***h***, multiplied by **tan (30)**, where 30° is the beam angle (Equation. 1).

(Equation 1) $$np = \frac{w}{2h * \tan(30)}$$

Artifacts in the data were sometimes a problem (Fig. 4) and difficult to differentiate with the Cobra-Tac supplied software (Surfer). It was necessary to post-process the data in Matlab in order to investigate apparent anomalies and remove artifacts, such as the extreme peak and pit seen in Fig. 4. The pit occurred in what we thought was a flat sandy bottom. The peak and pit formation were the result of the unit briefly tipping beyond level, allowing the unit to register far away points as an apparent bottom (Fig. 5).

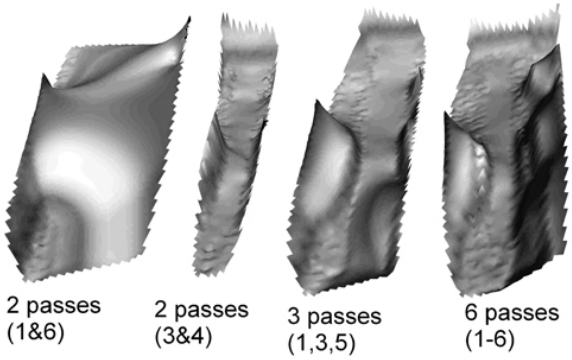

Figure 3: Surfaces created with different combinations of passes over the study site: passes sequentially ordered 1-6 going from west to east. 1 and 6 are the outermost passes of the 6 total.

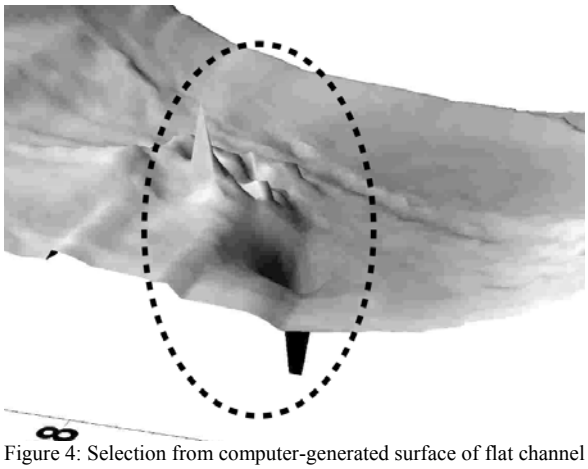

Figure 4: Selection from computer-generated surface of flat channel bottom, interpolated from bad data which resulted in anomalous structures visible within the dashed ellipse.

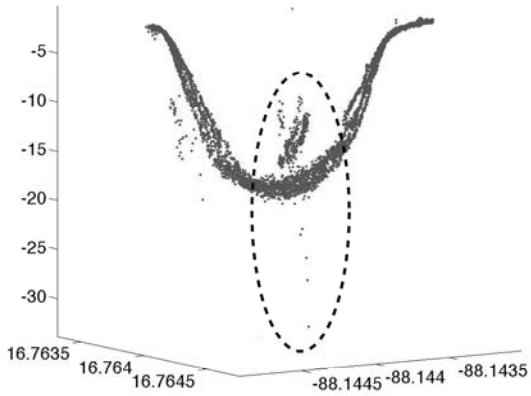

Figure 5: Matlab generated plot of cross channel survey data points as individual dots. Dotted ellipse indicates suspected outlier points, especially in lower portion of plot (below 20 m on z-axis).

Error in mapping can also result if all four of the Doppler beams do not record. The number of beams

used to collect data and the complexity of surface recorded is related (Fig. 6). Repeated transects over the same location allow for more complex surfaces to be defined up to the limit of the beam resolution. The beam resolution can be calculated, in theory, based upon the specification of a 1.4° beam width. For example, at a height of 10 meters above the bottom, the spatial resolution is 0.28 m^2.

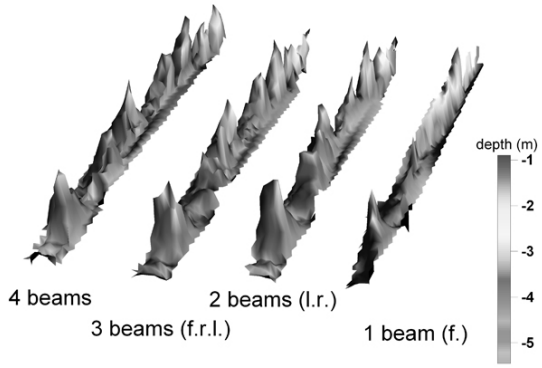

Figure 6: Surfaces created from 4, 3, 2 and 1 beams of the 4 beams on the Cobra-Tac. f= front, r= right, l= left. The complexity and accuracy of the 4 beams surface is significantly greater than that of the surfaces created from fewer beams.

Discussion

Cobra-Tac is a highly portable device that is capable of producing an accurate bathymetric map of a study site within 30 minutes of exiting the water. Waypoints of items of interest can be added while mapping underwater and displayed on the maps produced. The unit does not require a boat; therefore, it can be used in areas inaccessible to vessels. It can be towed behind a boat (with diver) but does not require any specialized apparatus beyond a towrope.

Cobra-Tac is not limited to working from the surface. This allows the device to be kept at a constant altitude above the bottom by a diver when moving over structurally complex habitats. Thus providing consistent resolution of bottom topography. The data are easily incorporated into GIS software, such as ESRI's ArcScene and ArcMap. With a luggage case for the unit and a laptop for data-processing, one can travel very easily with the Cobra-Tac.

The Cobra-Tac was able to map the bathymetry of local areas well. While the accuracy was fine enough to create bathymetric maps, our preliminary data were insufficient to assess if the Cobra-Tac will be a feasible tool for measuring fine scale rugosity (< 10 cm).

Data artifact creation is only avoided by maintaining vigilance while operating the unit and keeping it level. Data anomalies are easily investigated with Matlab, where a few outlying points can be recognized and removed. A shortcoming of the system is that the tilt sensor does not automatically filter data.

Overall, Cobra-Tac does create high-resolution bathymetric maps of reef areas. The effort of collecting and post-processing the data is simple and easy. A bathymetric map of appropriate resolution can provide a permanent record of the reef slope and medium to large-scale rugosity. Sampling over larger geographic scales (100's of meters) presents the opportunity for creating new metrics for habitat complexity at a landscape-scale. Such scales are more relevant for larger, commercially important fishes (Bartholomew et al. 2008). Additionally, Cobra-Tac is a non-invasive technique that does not require physical contact with the bottom or extraction of organisms or live substrate for laboratory measurement.

A device such as the Cobra-Tac has the potential for mapping changes in the structural complexity of reefs that may occur due to physical disturbance or coral mortality events. This is important, not only for monitoring the reef structure itself, but also because the loss of reef habitat results in decreased reef-associated fish biomass (West and Salm 2003). Such large-scale changes in reef structure have generally not been defined quantitatively over time due to technological limitations. Further field-work (in progress) will determine if the Cobra-Tac has the resolution and reproducibility to solve this challenge.

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Advancing spatial-temporal continuity in coral reef ecosystem pattern detection: The morphology, distribution and chemical environments of coral habitats encompassing Coiba National Park, Panamá.

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Abstract. A synoptic perspective of reef biogeochemical dynamics and community structure was revealed using new technologies and methods designed to enable high resolution underwater habitat assessment with non-invasive monitoring capabilities and rapid information output. A towed, chemical sensor platform (TETHYS) and a diver-based, benthic imaging system (SCUBA COP) were developed to compare reef architecture and seafloor morphology across centimeter to kilometer spatial scales, and resolve sub-meter variability in ambient water chemistry across 300 km seascapes. Acoustic bathymetry, stereo-optical imaging, *in-situ* underwater mass spectrometry, and fluorometry data were coupled with precision navigation to enable multi-parameter biogeochemical and structural comparisons of coastal and island coral habitats surrounding Coiba Island, a UNESCO World Heritage site in Pacific Panamá. Baseline chemical data [O_2, CO_2, CH_4, N_2]; and digital 3-D reef mosaics were coupled with SCUBA diver transect data, High Definition video, oceanographic time series from a cabled underwater observatory, Landsat and SeaWiFs satellite imagery to create and validate comprehensive, thematic chemical and benthic habitat maps. This integrated approach shows considerable promise for locating, predicting and quantifying natural and anthropogenic environmental stressors affecting the distribution, diversity and health of tropical coral communities.

Key words: coral reef ecology, benthic optical imaging, biogeochemistry, mass spectrometry, Panamá, Coiba

Introduction

Coral reefs are structurally intricate and complex biogeochemical systems existing in highly dynamic fluid environments. Efforts to link ecologic pattern to process in these variable and adaptive habitats requires precision, multi-scale, spatio-temporal information in order to establish quantitative baselines or accurately identify emergent or alternate stable states (Ratze et al. 2007).

Laboratory methods are limited in their ability to detect gross biogeochemical or metabolic changes of coral reefs over large spatio-temporal scales and may exhibit associated systematic sampling bias. Although SCUBA divers can intensively sample small areas *in-situ*, over brief intervals, physiological constraints makes replication costly (in terms of data density per diver-hour) and inherent information loss occurs through sacrifice in resolution when increasing observational domain (Lam et al. 2006; Hill and Wilkinson 2004). Limited *in-situ* capabilities generate sparse, spatially and temporally disconnected data sets with inadequate overlap, especially when used to corroborate larger scale processes or patterns (Garza-Perez et al. 2004; Mumby et al. 1999).

While coral reef deterioration along the Eastern Tropical Pacific (ETP) coasts of Panamá and Costa Rica were first identified nearly thirty years ago, understanding of the complex biotic-abiotic interplay which resonates and shapes coral communities within this Pacific biological corridor remains elusive (Glynn and Maté 1997; Guzman et al. 2004).

The focus of this manuscript is to convey new, ecologically relevant information emerging from the use of synoptic methods developed to quantify and characterize the architectural and chemical environments of coral communities across multiple temporal and spatial scales. Sites were chosen in the Gulf of Chiriquí (GOC) Panamá among island clusters and coastal zones which geographically delimit the current boundaries of the 216,543 hectare Marine Protected Area (MPA) of the Coiba Island National Park (Fig. 1).

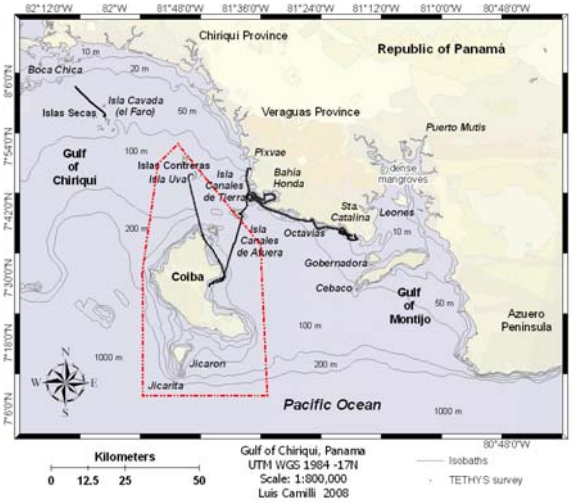

Figure 1: Pacific Panamá Gulf of Chiriquí (GOC) showing Coiba MPA boundary (red dashed line), coarse bathymetry, SCUBA dive sites, and TETHYS towfish survey (black line). Total TETHYS swath distance was 300 km with ≈ 36,000 discrete chemical measurements at 3 m spatial resolution (0.25 m - 15 m) deep.

Material and Methods

Towed, in-situ Mass Spec Chemical Surveys

In February 2007, real time, *in-situ* measurements of chemical species (i.e. O_2, CO_2, CH_4, N_2) chlorophyll-a (chl-a), chromophoric dissolved organic matter (CDOM), salinity, temperature and depth were accomplished using a towed chemical sensing platform equipped with a TETHYS underwater mass spectrometer, CDOM and chl-a fluorometers and a Conductivity, Temperature, Depth (CTD) sensor.

TETHYS was configured to monitor selected ion peaks corresponding to CH_4, H_2S, nitrogen isotope ratios (^{14}N -^{14}N and ^{14}N -^{15}N), O_2, Ar, and CO_2. Additionally, full spectral scans of 1-200 atomic mass units (AMU) enabled identification of hydrocarbons, industrial and toxic compounds. Mass spectrometer data were normalized to Argon which is biologically inert and serves as a natural tracer in ambient seawater. A single beam acoustic depth profiler collected bathymetric data while a Global Positioning System (GPS) device recorded position which was synchronized with TETHYS data based on internally logged time stamps (Camilli et al. 2007).

Average tow velocity was 2.5 m/s with each transect lasting about 6 hours. An oscillating depth or "Tow-Yo" approach was conducted by periodically slowing the boat velocity so that the towfish would perform a vertical cast, and increasing boat velocity until the instrument returned to its near surface depth (- 0.5 m). This technique created a sinusoidal profile of the water column at regular time intervals and was also employed when substantial change was detected in the surface waters.

Automated Diver Imaging Sled and SCUBA surveys

The SCUBA-diver operated Chemical Optical imaging Platform (SCUBA COP) is a self-contained dive sled that collects 12-bit, 1.4 megapixel video-rate stereo image pairs and simultaneously measures water depth, temperature, and dissolved [O_2]. Two laterally mounted and synchronized strobes minimize exposure time and variable effects of ambient lighting. SCUBA COP records mission start and end GPS surface position and continuously records dynamic vehicle trajectory and attitude (360° angular motion over 3 axes). Altitude above sea floor is triangulated based on stereo field of view (Camilli et al. 2007).

Seven dive locations were chosen to represent GOC areas with coral cover, and a stratified approach spatially randomized transects at each dive site using a list of random compass bearings and distances (10-100 m) from the dive vessel. Three, 50 m, tape-based linear transects per site were oriented perpendicular to shore to standardize swath coverage. Each transect was spaced a minimum of 20 m apart. Serial transects were captured at 2 m altitudes by one diver equipped with a Sony HDR-UX1 digital High Definition (HD) video camera followed by a second diver operating the SCUBA COP stereo imaging system. A third diver employing a Point Intercept Transect (PIT) method manually surveyed the same transects, defining 10 benthic categories at 0.5 m intervals below the tape (Reef Check 2004; Page et al. 2001).

Results

Reef Water Chemistry in the Gulf of Chiriquí

At an average tow depth of 2 m across the GOC, mean water temperature was 28.14 °C and salinity was 31.95 practical salinity units (psu) (N=35,953). Temperature and salinity for all coastal surveys were 28.7 °C and 31.85 psu, while offshore regions measured 29.9°C and 31.24 psu respectively.

Contrary to expectations, coastal transects over a shelf ≤ 30 m deep, homogeneously exhibited, lower temperature and higher salinity than offshore transects over deep water (≥ 50 m). The Panamá LJL Underwater Tropical Observatory (PLUTO), located at 18 m depth within the coastal survey area concordantly measured high frequency time series (≈10 min) oscillations in temperature ranging from 16° C to 28°C, and salinity fluctuations ranging from 27 to 32 psu over 3 m tidal amplitudes. Preliminary seiche calculations using measured wind velocity and water depth suggest that this effect is not a result of localized surface wind stress.

Low levels of dissolved O_2 were noticed between Canales de Afuera and Canales de Tierra islands and between the Islas Secas group to Boca Chica.

Comparatively high O_2 levels were observed near Isla Uvas and again northwest of Isla Coiba. Average [chl-a] for inter - island transects were higher 0.24 µg/L (+/- SD 0.02) than coastal transects 0.16 µg /L (+/- SD 0.06). The transect leg from Boca Chica to the Islas Secas group contained the highest mean [chl-a] 0.31 µg /L (+/- SD 0.05). Northwest along the coast, near the bay of Pixvae, chlorophyll was often near zero 0.05 µg /L (+/- SD 0.05) and low again going east along the coast until Santa Catalina where values increased to 0.20 µg /L (+/- SD 0.08).

Along the coast, CDOM concentrations averaged 5.50 Quinine Sulfate Units (QSU), and 2.87 QSU offshore. CDOM and Chl-a adjacent to offshore island reefs are shown in Fig. 2. Strong vertical gradients and localized anomalies dominated coastal regions of the Eastern GOC with CDOM reaching an order of magnitude greater (≥50 QSU) in the bay of Bahia Honda.

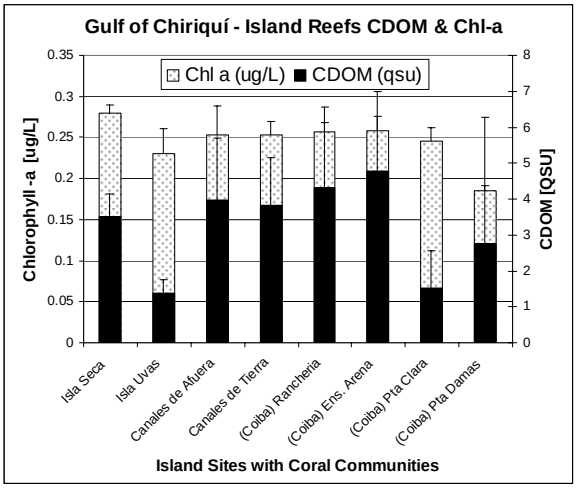

Figure 2: Comparison of mean chlorophyll-a [µg/L] and CDOM Quinine Sulfate Units [QSU] concentrations from TETHYS TowFish surveys of offshore islands in the Gulf of Chiriquí (≈15,000 samples). Error bars represent 1st standard deviation.

Survey transects provide evidence that the variability of nutrient mixing in these water masses can be extreme (Fig. 3). For example, a small plume of brackish water with salinity < 10 psu and high CH_4 was found subsurface near the coast of Santa Catalina (Fig. 4). Nitrogen isotopic (^{15}N) enrichment (i.e. low ^{14}N : ^{15}N) patterns were also observed in the western GOC between the Secas Islands and the Boca Chica mangrove mainland. A negative correlation between CO_2 and salinity (r = - 0.451; n = 842) suggests that freshwater input near island regions could be more important in determining CO_2 levels than pelagic water sources where carbon would ostensibly originate from atmospheric CO_2 drawdown. Offshore subsurface CDOM weakly correlates with salinity (r = + 0.245; n = 842). The reverse is true for the strong negative correlation (r = - 0.805; n = 260) between CDOM and salinity in the central GOC, supporting the idea that dissolved organic material input to these reefs may originate primarily from terrestrial sources.

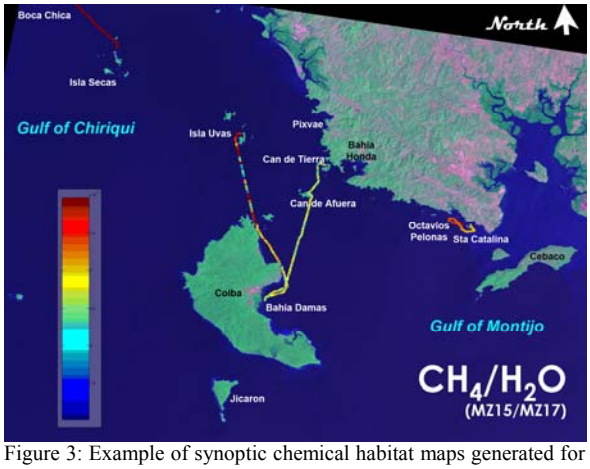

Figure 3: Example of synoptic chemical habitat maps generated for each bioactive parameter measured across survey areas. Color gradients indicate concentrations [mass (M) to charge (Z) ratios] of each element. Simultaneous chemical and GPS data were georectified and integrated into Landsat TM year 2000 satellite imagery to produce high resolution GIS composite images.

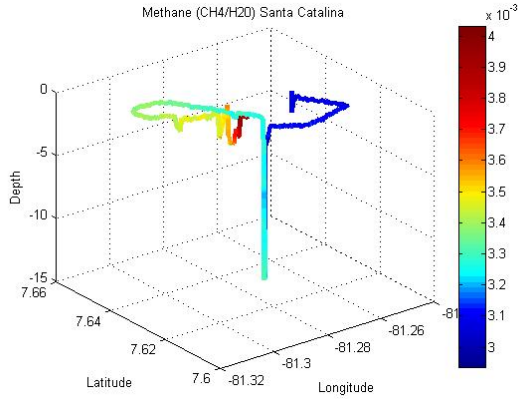

Figure 4: TETHYS sinusoidal profile and deep cast revealing (3-D) methane concentrations in the water column surrounding the island (and village) of Santa Catalina (reference Fig. 3). Color gradient areas in red indicate higher levels of methane (M/Z) and areas in blue indicate lower levels (dimensionless units). Depth = meters; latitude / longitude = degree-decimal-minute.

SCUBA surveys results

For the purposes of standardizing comparisons between methods, percent cover of each of ten categories was used to characterize the benthic substrate and sessile organisms across dive sites. The Reef Check method tends to overestimate sand cover and underestimate algal cover and coral rubble (Fig. 5). Both methods function similarly well in determining hard coral, silt, and rhodolith percent cover. Structurally complex, carbonate architectures dominated by *Pocillopora damicornis* and *P. Elegans* were encountered on northern (unexposed) shores of islands in shallow (<10 m) waters over a continuous

sloping shelf (i.e. Islas Contreras and Islas Uvas). More diverse, discontinuous and patchy coral communities were found on shallow rocky shelves on southern (wave exposed) shores. Soft coral, gorgonian and sponge communities were most often encountered in large boulder areas with swift currents ($\geq$ 15 cm/s) and colder water ($\Delta \geq$ -5°C) at depths 15 to 20 m. Hard corals, if present, existed in isolated patches closer to the surface, interspersed between heavily encrusted boulders. A filamentous red-brown algae (Cyanophyte) covered sandy areas (esp. Bahia Damas, Coiba) and stable sea grass communities were conspicuously absent in shallow sandy areas. Dense calcareous red algae (Rhodolith) beds covered shallow areas of some islands; (i.e. 40 % cover in Isla Canales de Afuera). Hard coral species encountered across dive surveys include members of Agariciidae: *Gardineroseris planulata, Pavona clavus, P. gigantea, P. varians, P. chiriquiensis*, *P. frondifera, P. maldivensis;* Pocilloporidae: *Pocillopora elegans, P. damicornis*, *P. capitata, P. eydouxi;* Poritidae: *Porites lobata, P. panamensis;* Siderastreidae: *Psammacora stellata;* Fungiidae: *Cycloseris curvata; Milleporadea*: *Millepora intricata;* Dendrophyllidae: *Tubastraea coccinea*.

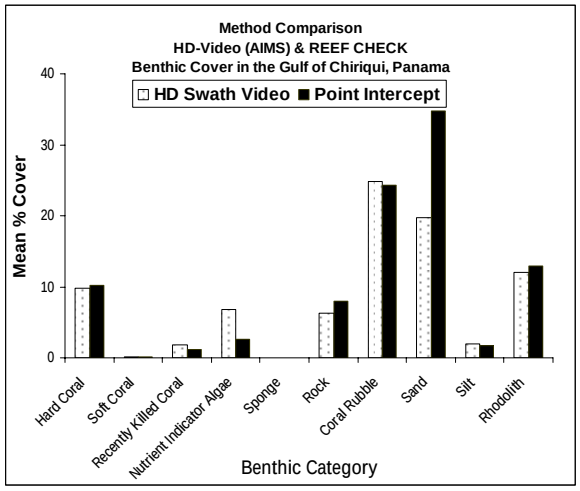

Figure 5: Reef Check (2004) and High Definition video (Australian Institute of Marine Science video protocol) using identical transects to assess 10 benthic cover categories across 7 dive sites in GOC.

The HD-video analysis (Fig. 6) indicates Isla Uvas and Isla Canales de Afuera with equally high coral cover (18.4%). Isla Canales de Tierra, near the mainland, was (16.6%). El Foro (Islas Secas) was (4.6%) - the lowest of all the offshore islands. Isla Managua located within the protected coastal bay of Bahia Honda, exhibited even lower (1.3%) coral cover when compared to Punta Miel (4.4%) at the western headland of the same bay.

Significantly more area was surveyed with the SCUBA COP than with HD video and PIT methods. Approximately 500 GB of digital stereo imagery were collected during 6 diving days corresponding to nearly 7 hrs of bottom imagery and 5 km linear data and representing 7500 m^2 area coverage. At this point, the mosaic swath processing can be divided into strips and applied to any subsection of the data ranging from 10s to 100s of square meters. Due to the fact that morphologically different coral species possess uniquely shaped surfaces, texture-based decision algorithms can be employed to automate coral classification from machine vision data. The subset of SCUBA COP mosaic swaths corresponding to the structured linear transects (i.e. PIT and HD video) are currently being used to create and validate automated feature segmentation and classification routines.

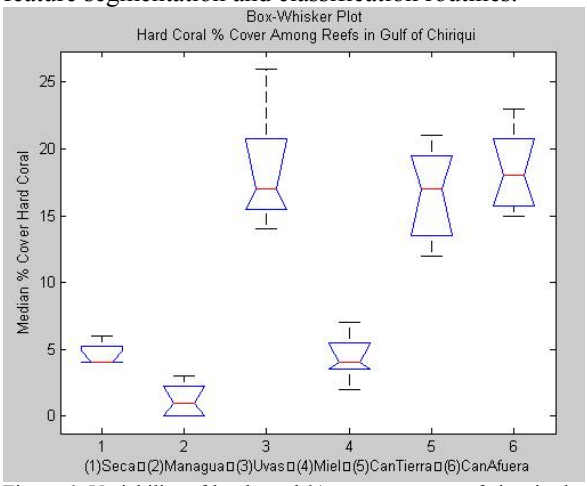

Figure 6: Variability of hard coral % cover among reef sites in the Gulf of Chiriquí. Lower and upper lines of each "box" = 25th and 75th percentiles. Distance between top and bottom = interquartile range. Horizontal middle line = sample median (skewness). Lines above and below the box = total sample extent. Notches in the box = confidence interval about the sample median.

Discussion

Multi-parameter TETHYS data imply that inter-island surface water chemistry (0-12 m) in the eastern GOC is relatively well mixed while shallow coastal areas are periodically exposed to cold water pulses during the dry season (boreal winter). Perhaps deep water originating in the adjacent Gulf of Panamá (or further offshore) is entrained in subsurface circulations that seasonally manifest as internal waves, eroding the thermocline and shoaling turbulently over shallow coastal bathymetry with added effect from orographic lift during large amplitude tides. Regardless of the mechanism, seasonal cold water pulses are regularly being delivered to coral communities, colder and more sustained than previously considered (*sensu*: Glynn and Maté 1997). Although these phenomena are likely influencing reef community structure and associated biochemical processes, unfortunately, subsurface mixing of cold water masses with warmer surface waters (≈10 m depth), and the spatial aliasing inherent in satellite sensors impairs routine remote

observation. The finer spatial-temporal resolution afforded by the TETHYS towfish allowed synoptic visualization of these winter, dry season temperature/salinity patterns and the associated primary productivity in subsurface water across a >100 km swath (Fig. 7).

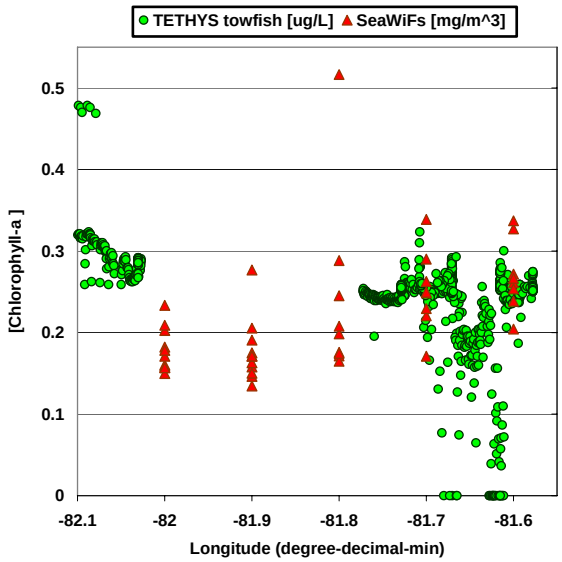

Figure 7: Comparison of *in-situ* TETHYS towfish and remotely-sensed SeaWiFs satellite [chl-a] data plotted along a longitudinal gradient during a time scale of 2 weeks in February 2007. Spatial resolution of SeaWiFs = 9 km^2 and finer spatial resolution of TETHYS towfish = 3 m^2. Data are reported in equivalent units [µg/L] = [mg/m^3]. Coarser SeaWiFs chlorophyll estimates provide an accurate upper limit constraint on sea surface chl-a, but are not able to substantially resolve smaller scale spatial-temporal dynamics over the same domain.

Contextually elevated CH_4, ^{15}N, CO_2, and [chl-a] coupled with lower O_2, suggests more eutrophic waters extending from the Boca Chica mainland into the central GOC. Although stretches of oligotrophic water were observed along the coast north and east of Coiba during this dry season survey, the risk of eutrophication along the Coiba Island corridor could be elevated especially during the wet season. Panamá experiences high seasonal rainfall (>3000 mm/yr) and these reefs could be severely impacted if corals are exposed to chronic runoff of nutrients originating from soil erosion, fertilizers or untreated sewage. Detrimental effects of NO_3^- enrichment (Schlöder and D'Croz 2004) in *P. damicornis* might be of great relevance for local conservation because it is the major reef-building coral in the ETP; a biotope known for intrinsically low coral diversity. GIS analysis of year 2000 Panamá Census data indicates >103,000 people inhabit the Gulf of Montijo drainage basin with 24 major tributaries eventually flowing into the eastern GOC (L. Camilli, unpub. data). Agricultural land use pressure, herbicides, erosion, unregulated coastal construction, mangrove deforestation, and increases in septic effluent in surrounding areas poses a tangible threat to the biological integrity of coral reefs ecologically linked with the Coiba MPA.

Coral reefs are complex adaptive systems that can experience accelerated habitat change due to their proximity to natural and anthropogenic disturbances worldwide. Semi-automated, *in-situ* chemical and optical sensor innovation reduce survey time and enhance the ability to effectively focus and tune experimental efforts. By resolving multi-dimensional ecologic and oceanographic variability in greater detail, the novel analytic methods demonstrated in this project complement remote sensing techniques, field manipulations, laboratory experiments, and become a critical nexus in accurately coupling pattern to process in the ocean environment.

Acknowledgements

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Is sponge bioerosion increasing?

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Abstract. We tested the hypothesis whether bioerosion is likely to increase on stressed coral reefs, using bioeroding sponges as a key group. A survey was conducted on the central Great Barrier Reef before and after two major bleaching events. While coral cover was slightly reduced, total bioeroding sponge abundances significantly increased from the first to the second survey (by 150%, $p < 0.001$). *Aka mucosa* became more common on the upper reef flat, and symbiotic bioeroding sponges proliferated on the lower reef flat and slope. Both groups are able to attack live coral. As typical after disturbance events, homogeneity of the sponge abundances was more pronounced after bleaching. Due to increased availability of settlement area and reduced space competition, we expect bioeroding sponge abundances and bioerosion rates to rise globally and would like to encourage more intensive field investigations on this group.

Key words: *Cliona*, *Aka*, bioeroding sponges, abundances, community shift

Introduction

On healthy reefs and under normal conditions, bioerosion is balanced or exceeded by carbonate accretion (Goreau and Hartman 1963), but this balance can be upset by shifts in environmental conditions or by disturbance events. As a consequence, reef-building organisms may suffer mortality, sublethal damage or stress (Berkelmans et al. 2004). In contrast, bioeroding organisms appear to continue to function normally or can occasionally even become epidemic (Rose and Risk 1985; Vicente 1990). Disturbance-related, changed conditions involve (i) the creation of suitable substrate for the settlement of larvae of bioeroding organisms, (ii) reduced space competition in favor of bioeroding organisms and (iii) reduced resistance of organisms invaded by bioeroders (Rose and Risk 1985). Considering that endolithic bioeroding organisms are relatively sheltered from otherwise adverse ambient conditions (Schönberg and Suwa 2007; Schönberg et al. 2008) and that many are filter feeders that may benefit from eutrophication (Rose and Risk 1985; Holmes 1997, 2000; Holmes et al. 2000; Ward-Paige et al. 2005), a rise in bioerosion appears to be a logical consequence if present trends of environmental deterioration continue. In extreme cases the positive balance of reef accretion will be overridden and bioerosion may prevail.

Sponges are the leading internal bioeroders on many coral reefs (MacGeachy and Stearn 1976). Many successful and aggressive species of bioeroding sponges harbor symbiotic dinoflagellates (Schönberg and Loh 2005), and have been reported to be more bleaching-resistant than corals (Vicente 1990). Reasons for this observation may stem from differences in the symbiosis of corals and bioeroding sponges: the 3-dimensional body of the sponges shelters symbionts from light more efficiently than coral tissue; the sponge G-type symbionts appear to be comparatively stress-resistant (Schönberg and Loh 2005; Schönberg et al. 2008); and the sponges are able to move the symbionts away from sources of stress (Schönberg and Suwa 2007). Recent observations have reported putative increases of bioeroding sponges in the Caribbean (Rützler 2002). However, to date we had no quantitative data on bioeroding sponge abundances both before and after a major disturbance event.

We report findings of a field study before and after bleaching at Orpheus Island, central Great Barrier Reef (GBR). The same survey was conducted in 1997/98 (Schönberg 2001) and in 2003/04. In 1998 and 2002 two major bleaching events significantly affected the study area, mainly killing branching corals (Berkelmans and Oliver 1999; Baird and Marshall 2002; Berkelmans et al. 2004). This presented an opportunity to investigate whether bioeroding sponges suffer from environmental changes as much as other reef organisms or, alternatively, whether bioeroding sponges tolerate environmental changes or even benefit from them. This study attempts to provide insights into possible future trends of reef bioerosion.

Material and Methods

The present study was conducted in the southern part of Little Pioneer Bay, Orpheus Island, central GBR (18°37'S and 146°29'E), mostly on SCUBA and partly by snorkeling. Methods were consistent in the first and second survey: Sponge occurrences were recorded along five 100 x 0.5 m belt transects perpendicular to the shoreline (see Fig. 2 in Schönberg 2001) using a 50 x 50 cm quadrat subdivided into 100 cm^2 squares. Any 100 cm^2 sub-square that contained bioeroding sponges was counted and then related to the amount of available calcium carbonate (see also Chiappone et al. 2007). Sponge occurrences are here represented as percentages per available substrate per square and then averaged over each reef zone (Table 1). Reef zones and sponge species were categorized as in the first study (Schönberg 2001), with the zones being defined as:

A) sand flat with only very little suitable substrate for bioeroding sponges, substrate often buried
B) mixed zone with sand patches, rubble, blocks and small live, massive corals with dead tops
C) coral pavement where mostly dead corals form a more or less continuous ground cover
D) branching coral zone, mostly reduced to rubble during the second study
E) reef relief, including live corals in patchy distribution

The sponge groups were:

1) the encrusting growth form of *Cliona orientalis* Thiele, 1900 (for information on growth forms see e.g. Schönberg 2008)
2) brown papillate bioeroding sponges with a papillar diameter > 2 mm (dominated by the papillate growth form of *C. orientalis*)
3) brown or orange papillate sponges with a papillar diameter < 2 mm (mainly including *Cliona caesia* (Schönberg, 2000) and *Pione vastifica* (Hancock, 1849), but to a smaller extent also *Cliona mucronata* Sollas, 1878 and *Cliona vermifera* Hancock, 1867)
4) yellow or orange papillate sponges with a papillar diameter of > 2 mm (*Cliona celata* Grant, 1826 *sensu* Schönberg 2000, *Cliothosa hancocki* [Topsent, 1888] and *Cliothosa aurivillii* [Lindgren, 1897])
5) *Aka* spp. (vastly dominated by *A. mucosa* [Bergquist, 1965] *sensu* Schönberg 2000, but also including the rare *A. paratypica* Fromont, 1993)
6) *Zyzzya criceta* Schönberg, 2000
7) other, comparatively rare bioeroding sponge species

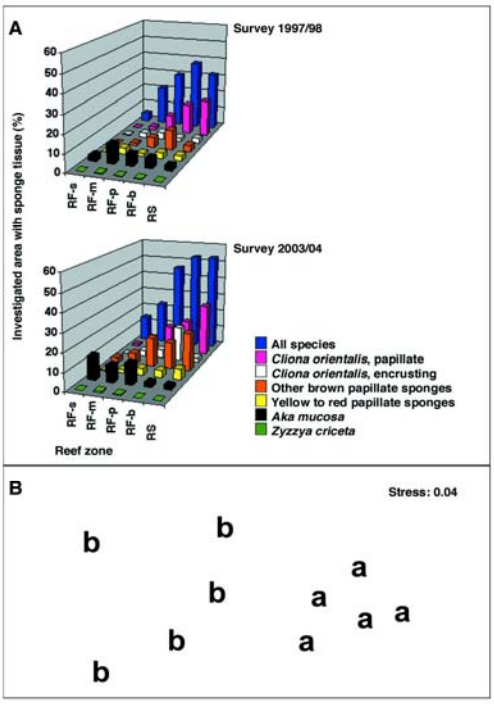

Fig. 1. Bioeroding sponge distributions at Orpheus Island. A: Increase in bioeroding sponge abundances on the central Great Barrier Reef. Abundances and distributions of bioeroding sponges are displayed for the different reef zones during the surveys 1997/98 and 2003/04. Reef zones: RF – reef flat, s – sandy, m – mixed, p – pavement, b – branching corals, RS – reef slope. During the second survey, dead substrate available for sponge settlement was +4, +1, -10, +8 and +11% in the 5 zones, respectively (Table 1). B: Changes in the community of bioeroding sponges represented by multidimensional scaling after an analysis of similarities (MDS; ANOSIM), b – before, a – after bleaching events (5 belt transects 100 x 0.5 m).

Substrate categories were distinguished as:

i) calcium carbonate in form of dead coral, mollusk shells etc.; suitable substrate for bioeroding sponges
ii) substrate covered with live tissue (corals, soft corals, other invertebrates, dense cover of algae); unsuitable substrate for all bioeroding sponges except for *C. orientalis*, which invades such areas laterally (Schönberg and Wilkinson 2001) and *A. mucosa* that may even be able to settle on coral tissue (Rützler 1971)
iii) sand; unsuitable substrate for bioeroding sponges (except for *A. mucosa* that is able to live in a piece of calcium carbonate buried in the sand; Schönberg 2001)
iv) mud; unsuitable substrate for bioeroding sponges (except for *A. mucosa* that is able to live in a piece of calcium carbonate buried in the mud; Schönberg 2001)

Where reference samples were taken with hammer and chisel, preparations were made according to standard procedures (e.g. Schönberg 2000) and included in the reference collection of the first author. Resulting species descriptions can be accessed through other publications (Schönberg 2000; Schönberg 2001; Schönberg et al. 2006; Schönberg and Tapanila 2006; Schönberg and Beuck 2007).

The earlier study was conducted 1997/98 (Schönberg 2001), the later study 2003/04. The first survey yielded data for a putatively largely undisturbed situation. The more recent survey took place two years after two severe bleaching events, which had a significant impact at the sample site and caused severe disturbance that partly resulted in over 60% coral mortality (Berkelmans and Oliver 1999; Baird and Marshall 2002; Berkelmans et al. 2004). To date only the survival of reef builders had been surveyed in this area, but not the survival of bioeroders. The studies reported here represent an unprecedented before-and-after investigation and focused on the question whether bioeroding sponges had suffered to the same extent as corals. 'Before' data were compared with 'after' data with multidimensional scaling for community composition using analysis of similarities (MDS; ANOSIM), and for total area of bioeroding sponges per survey using a paired t-test.

Table 1. Material composition and bioeroding sponge abundances averaged over all survey transects in Little Pioneer Bay, Orpheus Island comparing the situation of 1997/98 with 2003/04. Values in parentheses are standard deviations.

Zone	Material [% transect area]								Sponge abundances			
	Calcium carbonate		Live tissue		Sand		Mud		[% transect area]		[% substrate area]	
	1997/ 1998	2003/ 2004	1997/ 1998	2003/ 2004	1997/ 1998	2003/ 2004	1997/ 1998	2003/ 2004	1997/ 1998	2003/ 2004	1997/ 1998	2003/ 2004
Sand	41.7	45.9 (20.0)	2.0	3.3 (2.1)	56.3	50.8 (20.1)	6.7	0.0 (0.0)	1.9 (2.0)	6.1 (4.3)	4.6	16.8 (13.9)
Mixed zone	70.7	71.5 (6.4)	11.7	12.4 (7.5)	17.6	16.0 (9.9)	9.3	0.2 (0.4)	8.4 (2.6)	9.3 (8.4)	11.9	13.1 (12.2)
Coral pavement	84.2	74.1 (5.8)	15.5	20.2 (5.4)	0.3	5.2 (3.9)	18.9	0.5 (0.5)	12.4 (1.6)	18.9 (2.41)	14.7	25.7 (3.7)
Branching corals	62.9	71.2 (9.0)	36.4	22.8 (5.8)	0.7	5.5 (8.0)	18.8	0.5 (0.9)	13.8 (10.8)	18.8 (7.64)	21.9	27.0 (7.2)
Reef relief	57.5	68.8 (6.7)	32.5	22.7 (9.0)	0.0	0.2 (0.3)	20.7	8.6 (6.2)	12.6 (2.9)	20.7 (6.2)	21.9	29.6 (11.0)

Results and Discussion

Both, the 1997/98 and the 2003/04 survey on bioeroding sponge abundances on the central GBR showed that symbiotic bioeroding sponges dominated over non-symbiotic ones (most common: *Cliona orientalis* and *Cliona caesia*; Fig. 1; latter as *Pione caesia* in Schönberg 2001). Another very common sponge was *Aka mucosa* that can survive on the innermost reef flat and in the mud behind the reef crest, its body being buried in the sediments and cm-long fistules reaching into the water column. *C. orientalis* and *A. mucosa* can attack live corals, while most other bioeroding sponges can only settle on or expand into dead substrates (Rützler 1971; Schönberg 2001; Schönberg and Wilkinson 2001).

Between the first and the second survey (Fig. 1A) a clear escalation in bioeroding sponge abundances occurred, with an overall increase of total bioeroding sponge area of about 150% ($p < 0.001$, paired t-test), and a stronger prevalence of *A. mucosa* on the upper reef flat and the symbiotic bioeroding sponges on the lower reef flat and on the reef slope. For *C. orientalis*, especially the early, papillate growth form became more common, indicating that larval settlement continued unhindered or was even enhanced after the bleaching events by increased availability of suitable substrate (Table 1). Despite clear evidence of the demise of various species of corals and soft corals, the more mature, encrusting growth form of *C. orientalis* had also increased in the zone formerly inhabited by branching corals, suggesting that established colonies of this sponge did not die during the bleaching events, but spread in reef areas where most coral mortality occurred. Using multidimensional scaling, mean bioeroding sponge areas per transect and available substrate clustered separately for both surveys and differed significantly, with the cluster from the first survey more dispersed than the cluster from the second survey (Fig. 1B; ANOSIM $r = 0.804$, $p = 0.008$). This pattern indicated that sponge community composition became more homogeneous, possibly implying the occurrence of a major disturbance (Connell and Keough 1985). We ascribe this disturbance to the recent bleaching events, resulting in an increase of dead surface area available for sponge larval settlement (Table 1) and reduced space competition by other reef organisms (see also Chiappone et al. 2007).

The present study represents a localized survey, but is supported by other reports of increases in bioeroding sponge abundances (Rose and Risk 1985; Rützler 2002). If we accept that bleaching events were the cause for this shift, then we will have to expect globally increasing bioerosion rates on coral reefs. This prognosis appears even more likely when considering that many sponges may benefit from growing eutrophication and pollution (see e.g. Holmes 1997, 2000). We would like to encourage further field studies on bioeroding sponges, and preferably investigations over time.

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The Semantic Reef: A hypothesis-based, eco-informatics platform to support automated knowledge discovery for remotely monitored reef systems.

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Abstract. Eco-informatics is the combination of multiple environmental datasets and modeling tools used to test ecological hypotheses and derive information. The Semantic Reef project is developing automated data processing, problem-solving and knowledge discovery systems to better understand and manage reef ecosystems. Three core tools are utilized; Semantic Web, Grid Computing and workflow based e-Research approaches, creating a platform designed to evaluate complex hypothesis queries and provide alerting for unusual events (e.g. spawning, bleaching). Remote environmental monitoring is being widely deployed to gather data in real-time. As the volume of raw data increases, bottlenecks are expected to develop in the data analysis phases - current data processing still involves human intervention and manual manipulation. Semantic Web technologies address this data deluge through using explicit descriptions, called ontologies, of the datasets and structures, making the data machine-understandable, therefore automating data integration and processing. The Semantic Reef project is focused on developing reef ontologies, which, when coupled to datasets, derives inferences from data to 'ask' the system questions for semantic correlation and analysis. Currently, the model is being extended to map dynamic data from reef-based sensor-networks into the ontology in real-time, offering a new approach to solving problems of scale across reefs.

Key words: Eco-informatics, semantic web, ontologies, coral reefs.

Introduction

With the global effects of climate change, and other major issues, eco-informatics is an emerging branch of research where new techniques, tools and infrastructure are being developed to enable far greater scope for problem solving methodologies and analytical ability for the research scientist. This new cross-discipline evolution of global collaborations, with both ecological and computer scientists, is vital in the quest for knowledge and answers when addressing such major issues as climate change.

Problems are arising, however, with the imminent influx of new data and information, appropriately dubbed 'the data deluge'. This increasing flood of data is growing exponentially with the large number of deployed, or soon to be implemented, scientific data collection instruments such as sensors, satellites, scientific experiments and simulations. Hence, as the volume of raw data increases, bottlenecks in the data processing and analysis phases are occurring because current methods still involve human intervention making it progressively more difficult for the scientist to keep up effectively.

The Semantic Reef Project aims to utilize existing coral reef databases, augmented by real time sensor output, to pose hypotheses of the disparate data. The project is a Knowledge Representation (KR) system that employs Semantic Web, scientific workflows and Grid computing technologies to resolve the problems of data integration, synthesis and discovery for coral reef ecosystems (Fig. 1). It will map static and dynamic data to a set of ecosystem ontologies, which through explicit definitions will make the information machine-understandable, enabling the computer to make intelligent inferences, decisions and/or

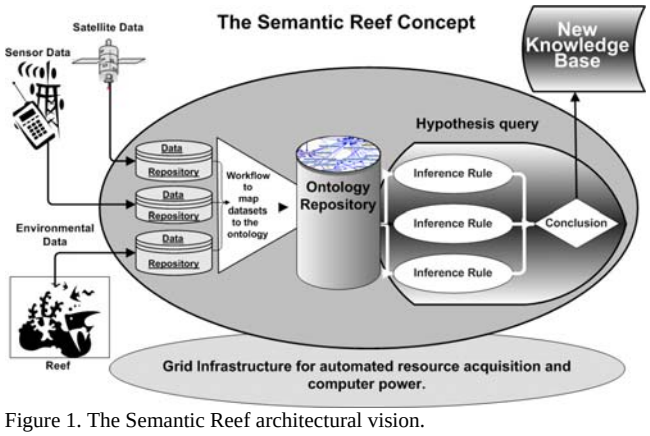

Figure 1. The Semantic Reef architectural vision.

discoveries, using logic systems such as Description Logics (DL) and propositional logic (Myers et al. 2007a).

A brief description of the Semantic Reef project, results to date and the data processing issues marine biologists are facing will be followed by a closer look at Semantic technologies, and how they may address this data deluge problem. Finally, details of the KR system and the reusable design methodologies, to semantically represent a full coral reef ecosystem, will be explained.

Background – The Semantic Reef project

The Semantic Reef project is developing a tool for use in hypothesis-driven research and problem-solving methods. It will allow for automated data processing and analysis of disparate databases and data streams through the technologies enlisted in the architecture. These technologies include Semantic Web, Grid computing and scientific workflows, which together create synergies to address particular issues in data integration. The resulting technology platform is designed to improve our capacity to generate timely warnings of environmental conditions that are conducive to climate change issues or unusual events within the coral reef environment.

At the heart of the Semantic Reef project are the Semantic Web technologies, which are an emerging area of computer science that aims to support automated processing of information. Through ontologies, which are the foundation of Semantic technologies, concepts are explicitly described, giving context and meaning to the data the computer can access. Here, a set of re-usable ontologies have been developed to describe to a computer the concept of, and the relationships within, a coral reef ecosystem.

The model is built on a Grid Computing foundation which provides the tools for secure and reliable access, sharing and management of resources, namely raw data and computational power (Foster et al. 2001). The scientific workflows are developed using the Kepler workflow software (Altintas et al. 2004), to automatically process raw data, and pass the results through the expert system.

To assess the accuracy of the architecture, validation tests were conducted. Initially, with a focus on the coral bleaching phenomena, a reverse hypothesis methodology to ground truth the system was employed and the outcome of the inference propositions were compared with actual historic bleaching events. For the validation, the stress factor most commonly associated with the bleaching event, namely elevated sea temperature (Marshall and Schuttenberg 2006), was used with the available historical data acquired through the Great Barrier Reef Marine Park Authority (GBRMPA) on the 1998 and 2002 mass bleaching events. The thermal stress indices trialed were the Sea Surface Temperature plus (SST+) and Degree Heating Days (DHDs), which are commonly used to describe thermal anomalies (Berkelmans et al. 2004; Maynard et al. 2008). Logical inference rules and DL were used to mimic these metrics then executed using the historic temperature data. The results of the automated inference were found to relate closely to those of previous research on the tolerance of corals to temperature changes, thus verifying a successful test (Myers et al. 2007b).

Since the initial validation exercise, a richer set of ontologies have been developed. The hierarchical design employs re-usable multi-scale ontologies to represent the functionality and complexity of a coral reef ecosystem making the concept computer-understandable for auto-processing objectives. The intention is to allow marine scientists, from around the globe, tools to test hypotheses and probabilities, through enlisting a semantic 'richness on demand' environment. The ontologies, ranging in complexity, describe concepts such as the composite population of the reef community, human influence contributors, bathymetry, environmental factors, among others, in computer-understandable semantics. Using this modular approach, reusability is afforded, where the knowledge being sought or the hypothesis being posed will determine the choice of which ontologies to utilize and what data is used to populate them. For example, extending the validation inference rules used for a bleaching occurrence, which are currently based solely on SST, to introduce other premises such as water quality, location, nutrient levels, salinity, etc, would be a trivial task programmatically.

The Data Deluge

Many scientific disciplines are experiencing changes in how research is being performed due to digital technology from the next generation of experiments, simulations, sensors and satellites generating a flood of valuable data for scientists to interpret (Hey and Trefethen 2003). The data is being gathered globally in differing formats and for different agendas and the bridging between all these disparate sets is the real issue that many technological developments, such as the one described here, are attempting to address.

The use of environmental sensor networks to gather data in real-time across widely distributed areas is an expanding field, detailed by projects such as the Integrated Coral Observing Network (ICON) in the USA (NOAA-ICON/CREWS 2008) and the Integrated Marine Observing System (IMOS) in Australia (IMOS 2008). Applications of new technologies and processing systems are being trialed on the Great Barrier Reef (GBR) such as the Great

Barrier Reef Oceans Observing System (GBROOS 2008). As habitat researchers deploy embedded sensor networks, strategies on capturing, organizing, and managing large amounts of streaming live data is becoming a critical issue. It is imperative the processing and analyzing of the data be effective and efficient. However, it is becoming evident that current technologies are not scalable enough to quell the imminent deluge or make proficient methods of analysis and research possible.

The data is being gathered and stored in a myriad of different repositories and although there are data mining efforts to bridge across this disparate data most are still considered stand-alone unconnected data silos. So how do we search and infer new knowledge across the breadth of available data automatically? With the main focus of data accessibility, integration and automated processing, Semantic Web technologies offer a possible solution.

Semantic Web Technologies

Semantics is the study of meaning and dates back millennia in the philosophical arena. The term 'Semantic Web' was coined by Sir Tim Berners-Lee in his original proposal to CERN to develop the World Wide Web (Berners-Lee 2000). He described the Semantic Web as being the evolution from a Web of cross platform documents to a 'Web of data', where complex decision making by the machine will be possible as the information contained within the web pages will be both human-readable and computer-understandable. Ideally, with the decrease in manual intervention, it will allow search and analysis mechanisms greater autonomy to sift through the massive amounts of knowledge and data available on the Web and automate the process of creating new knowledge.

The Ontology

At the heart of Semantic technologies is the ontology. Ontologies are documents or files that formally define the relations among terms and representing abstract or specific concepts: intentions, beliefs, objects or feelings. These descriptions contain explicit specifications, terms and relationships with formal definitions, axioms and restrictions that constrain the interpretation by the computer, thus making data computer-understandable enabling it to make intelligent decisions based on inference rules and DL (Antoniou et al. 2001). Of course, the computer does not literally 'understand' the information, but it can now manipulate the terms much more effectively in ways that are useful and meaningful to the human user, such as being more functional in cross platform environments when dealing with disparate data from diverse sources.

Modern ontologies can be engineered at differing levels of complexity, from shallow (i.e. a domain vocabulary, thesaurus, or taxonomy) to the composite category, which involves applications of logical definitions to automate conclusions, assumptions and subsumptions through classification and inferences. Creating unambiguous definitions of things, concepts and ideas assists in bridging across disparate data sets, for example, well defined synonymous relationships would allow '*Acropora formosa*' in one database schema to be easily bridged to 'stag horn coral' in another. In addition, defining homonyms by adding context affords the ability for the computer to interpret contextual information. For example, the word 'fluke', to the machine, is five characters of eight binary bits each, it has no meaning to the computer, however, associating the word with a 'part of' or an 'is a' relationship to another word, such as anchor, dolphin, fish or flatworm, adds enough contextual information so the computer can make meaningful connections automatically.

The Logic Systems

There are number of differing formal logic systems available within the scope of Semantic technologies each orthogonal to the next. The field of DL is a subset of First Order Logic (FOL) and has been used throughout history, it allows for expressing relations between concepts in a generalized fashion for reasoning with logical axioms and quantifications (Baader 2003). To give a simple example, an omnivore could be generally defined as, among other things, any animal that eats both plants and animals. Translated into the Web Ontology Language with DL (OWL-DL) (McGuinness and van Harmelen 2004) would be written as:

```
OmnivoreClass eats SOME
(PrimaryProducerClass AND
(HerbivoreClass OR CarnivoreClass))
```

Upon classifying using a reasoning engine, any instances that are defined with the property 'eats' and is linked to both a member of a plant class and animal class (e.g. an instance of the Sea_Grass class and the *Bivalvia* class respectively), will automatically be subsumed to belong to the omnivore class (e.g. all instances of the Loggerhead_Turtle class). This ability of the KR system to automate latent connections and make dynamic inferences of relations (i.e. automatically connecting the hidden dots), is imperative for modeling such an intricate multi-scaled concept as a reef ecosystem.

Propositional logic adds the ability to infer conclusions based on sets of predefined premises. The Semantic Web Rules Language (SWRL) uses 'horn-like' rules, composed as syllogisms, to produce logical conclusions (Horrocks et al. 2004), which

allow for hypothesizing over the full knowledge base. Posing hypothetical questions with known or best guess factors can allow for conclusions to be drawn by deductive and inductive inference, where observation would prove or disprove the hypothesis.

The Open World Assumption

Marine science, in general, maintains an Open World Assumption (OWA), that is, nothing is false until explicitly proven false. Traditional database implementations such as relational databases are required to maintain a Closed World Assumption (CWA), that is, everything that is known about the world exists within the boundaries of the database and its schema. This creates a mismatch between the researchers need for dynamic multi-scale complexity open to changes upon new discoveries and the current technological capabilities for flexibility.

Semantic Web languages have an OWA where it is assumed knowledge of the world is incomplete. Specifically, 'not true' is not automatically false, it is considered unknown; it assumes the extra information required has not yet been added to the knowledge base. As new discoveries are quite feasible in the marine biology domain, there is a need for flexibility allowing new knowledge and concepts to be assimilated into the system without difficulty; the OWA caters to this need (Horrocks et al. 2003).

In illustrating the differences between OWA and CWA, when describing the makeup of a particular reef the schema includes only these two statements: 'Davies Reef has *Favites*' and 'Davies Reef has *Porites*', a query of whether Davies Reef contains any *Acropora* would return false in a CWA, which would be incorrect. However, with the OWA, unless there is a statement that explicitly declares 'Davies Reef has no *Acropora*', the system will conclude there may be *Acropora*, it simply has not yet been explicitly asserted.

Building the Semantic Reef ecosystem ontologies for multi-scalability

Concepts can be modeled through ontologies in a variety of ways and varying degrees of granularity. The Engineering and design choices on what type of ontology to build is determined purely by the degree of extensibility and expressiveness required to produce the desired information or knowledge. However, the flexibility and reusability becomes more restricted, in ontological design, as complexity increases (Gomez-Perez et al. 2004).

Clearly, coral reefs are highly complex, interdependent ecosystems. It became apparent the use of a singular top-down, large ecosystem ontology would not be simple to create, implement nor maintain, as the number of variables and multi-scale relationships are immense. Therefore, a 'mix and match' bottom-up approach in the ontological design was adopted. Specifically, a range of separate ontologies were created to describe the many diverse concepts that make up a coral reef, such as, community and environmental composition, hydrodynamics, human influence and trophic layers, among others.

The current set of ontologies consist of reef and environmental taxonomies at the lowest layers, which import to heavier-weight DL ontologies for concepts such as trophic layers that require richer relational descriptions (Fig. 2). To illustrate, the reef community composition is an uncomplicated ontology, where only synonymous and hyponymous (i.e. 'same as' and 'is a') relations are defined. When populating the ontology, this method allows for a less complicated bridging mechanism across disparate databases, which may list either scientific names or common names, but not both. This and other simplified ontologies, such as one that describes and maintains temperature information, can then be ported to the heavier-weight ontologies. Separating the logical complexities from the instance data at the lower layers, makes ontological structure reusable.

'Domain task specific' ontologies lie at the highest level of granularity. It is at this level the finely detailed rules are introduced as propositions, written in SWRL, to infer conclusions from the available data within the knowledge base. For example, to hypothesize about coral bleaching on the GBR would have all lower ontologies populated with available or relevant data (e.g. salinity, chlorophyll, real-time temporal and spatial SST data, etc), then imported into a domain task GBR ontology, where inference

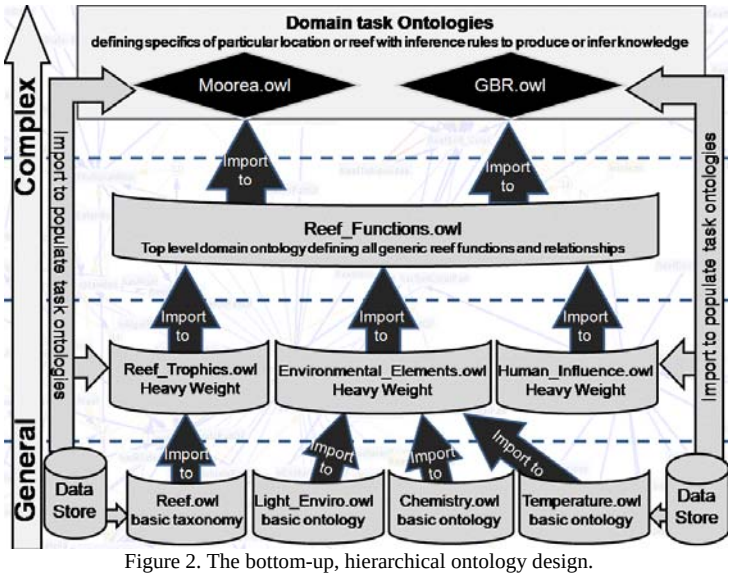

Figure 2. The bottom-up, hierarchical ontology design.

rules can be structured to infer a bleach alert. As the infrastructure is designed to be generically reusable, the same ontologies, populated with different location specific data, could be used for a different hypothesis on any reef, for example, a sensitivity analyses for coral spawning on Moorea Reef (Fig.2).

Future Works and Conclusions

We are drowning in a sea of data, which occasionally is generously referred to as 'information' and almost all of it must be interpreted by humans to be of any use. The growth and availability of new data sources and, therefore, our need to consider it in research, decision-making and planning is growing exponentially, and our systems, rather than helping with this, are predominantly contributing to the problem. Hence, the demand for automated data analysis and/or hypothesis-testing systems is becoming increasingly imperative as the escalating range of data gathering devices and instruments are deployed.

The Semantic Reef is a new approach to such data analysis and interpretation issues. The modular ontology design allows for inclusion of both scientifically known factors as well as phenomena yet to be discovered. Therefore, as user driven propositions change due to new findings, information or queries, and as new data sources become available, the open world nature of Semantic technologies will make uncomplicated additions and eliminations to the KR system possible.

Here, highly diverse backgrounds and expertise have combined effectively in collaboration to structure a semantically driven architecture to assist the marine biology domain in confronting the data deluge challenge. Currently, the processing capabilities of the Semantic Reef system are being tested to infer a coral bleach warning using satellite data from the US National Oceanic and Atmospheric Administration (NOAA 2008) and real time SST data streamed directly from the Davies Reef microwave site, part of the GBROOS project (GBROOS 2008). Further additions to the hypothesis will see the addition of other causal factors, as the data becomes available, such as chlorophyll provided by AIMS and salinity levels from IMOS.

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GBROOS – An Ocean Observing System for the Great Barrier Reef

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Abstract. The Great Barrier Reef Ocean Observing System (GBROOS), a geographic node of the Australian Integrated Marine Observing System (IMOS), is an observation network deployed along the Great Barrier Reef (GBR) in Northern Australia. The project aims to quantify and monitor the impact of the Coral Sea, in particular cool and warm water intrusions, on the GBR and to provide the real-time data required to understand the impact of climate change and other environmental factors on coral reef ecosystems. The project has five components. Sets of paired deep (200 m) and shallow (30-70 m) oceanographic moorings will be deployed to detect water moving onto and along the GBR from the pole-ward East Australian and the equatorial Hiri western boundary currents. Upgraded Remote Sensing capacity, coupled with underway validation data, will give large-scale information about the GBR. A set of reference moorings will provide long-term baseline data on water parameters supplemented by detailed monthly water samples. Sensor networks located at seven sites will provide real-time information about small-scale phenomena. The observational data will have significant impact on our understanding of global change, its potential impact on the physical and chemical conditions and the associated changes to the biology and structure of the GBR.

Keywords: GBR, Ocean, Observing, Sensor networks, Climate change, Great Barrier Reef

Introduction

GBROOS is an observation network that seeks to understand the influence of the Coral Sea on continental shelf ecosystems in north-east Queensland including the GBR Marine Park.

The South Equatorial Current (SEC) is the dominant flow in the Coral Sea. On reaching the Australian coast, the SEC bifurcates into northern and southern boundary currents. The northern arm is responsible for driving a clockwise gyre in the Gulf of Papua that is a nursery for tropical rock lobster; a major resource for indigenous communities in the Torres Strait. The southern arm becomes the East Australian Current (EAC), which flows down the eastern seaboard and affects coastal climate and ecosystem performance from southern Queensland to Tasmania (Ridgeway and Dunn 2003).

The SEC is dynamic on annual and decadal time scales. Variations in flow of the EAC associated with the Southern Oscillation Cycle (El-Niño/La-Niña) affect the thermal and carbonate chemistry regimes on the outer barrier reef, and the replenishment of commercial fish stocks along the eastern seaboard (Harris et al. 1988; Oke and Middleton 2001).

In the central GBR, the slope bathymetry favours intrusion of Coral Sea water onto the continental shelf and flushing of the outer Lagoon while also suppressing cross-shelf exchange. Many of these intrusions draw cool nutrient-rich water from the deeper Coral Sea onto the shelf (Andrews and Gentien 1982).

The Coral Sea has a direct impact on the water that is delivered to outer-shelf reef systems and to in-shore areas where intrusions are able to penetrate through the reef matrix. These incursions play a critical part in determining the water that forms the lagoon of the GBR (Steinberg 2007). The large scale circulation and characteristics of oceanic water influences local factors and circulation to determine the thermal and other characteristics of the water that the corals experience. In order to understand how corals respond to changes in their environment we must first measure that environment, and for the GBR, this means understanding the impact and functioning of the oceanic water processes.

Material and Methods

There are five components to GBROOS that together provide information at a range of scales from synoptic remote sensing data to fine scale sensor network data.

The remote sensing component involves the installation of an L and X band receiving station in Townsville for the collection of the older AVHRR data and new higher resolution data such as MODIS. This compliments an existing L-Band receiving station that has been operating since the mid 1980's.

To compliment the remotely sensed data a number of vessel-based systems are deployed to collect daily validation data. A radiometer mounted on a commercial ferry that transits between Shute Harbour and Hardy Reef collects radiance data to validate the AVHRR SST data. An Ocean Colour reference station is being established near Lucinda in North Queensland to validate the MODIS ocean colour data.

Underway water quality data is being collected from two AIMS research vessels (the *R.V. Cape Ferguson* and the *R.V. Solander*) and a commercial ferry operating between Gladstone and Heron Island in the southern part of the Great Barrier Reef. The instruments consist of a Sea-Bird thermo-salinograph (temperature and salinity) and a WetLabs FLNTU (chlorophyll and turbidity) as well as position from the vessel GPS. The data is downloaded nightly and will be used to validate other data, such as remote sensing data, and to build up a long-term data series. For the commercial ferry this will be for the same transect, for the research vessels this will be for their area of operation over northern Australia.

As part of the larger IMOS project a number of identical reference moorings are being located around Australia to provide equivalent long-term records of basic water parameters. GBROOS is responsible for two of these moorings; one off Townsville in North Queensland and one near Darwin in the Northern Territory. The instruments deployed include a near-surface (around 10 m depth) and near-bottom (around 5 m from the bottom) WetLabs WQM (temperature, salinity, depth, turbidity, chlorophyll), a series of temperature sensors (Sea-Bird SBE39's) along the mooring wire, a surface buoy mounted weather station (Vaisala WXT-510/520 – air temperature, wind speed and direction, relative humidity, rain duration and amount, barometric pressure) and waves and currents (via a bottom mounted Nortek AWAC). The design is shown in Fig. 1.

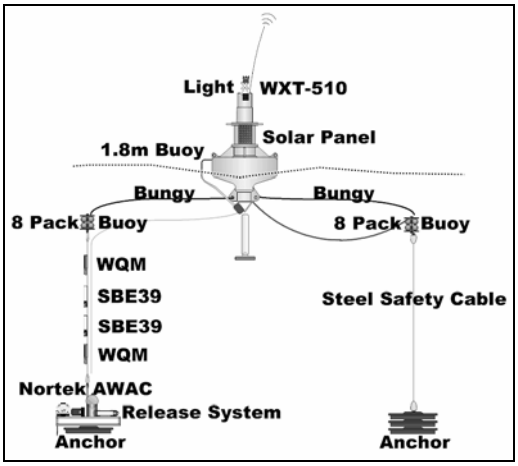

Figure 1: The basic design of the Reference Station moorings.

Water samples will be collected from the Reference Stations monthly. The parameters investigated include hydrochemistry (nitrate, nitrite, phosphate, etc), carbonate chemistry, suspended matter, zooplankton, phytoplankton pigments, as well as a CTD cast and validation samples for the instrument data.

The fourth component of GBROOS is the GBR Mooring Array. This consists of four pairs of moorings located along the GBR with one of the pair located on the continental slope in relatively deep water (70-200 m) and the other of the pair located on the continental shelf in shallower water (30-70 m).

The design looks to detect the movement of oceanic water up the continental slope and onto the shelf and so give information about upwelling events and about the strength and direction of the oceanic currents as they impinge on the reef matrix. The locations of the moorings and the main current flows, as measured by surface flows, are shown in Fig. 2.

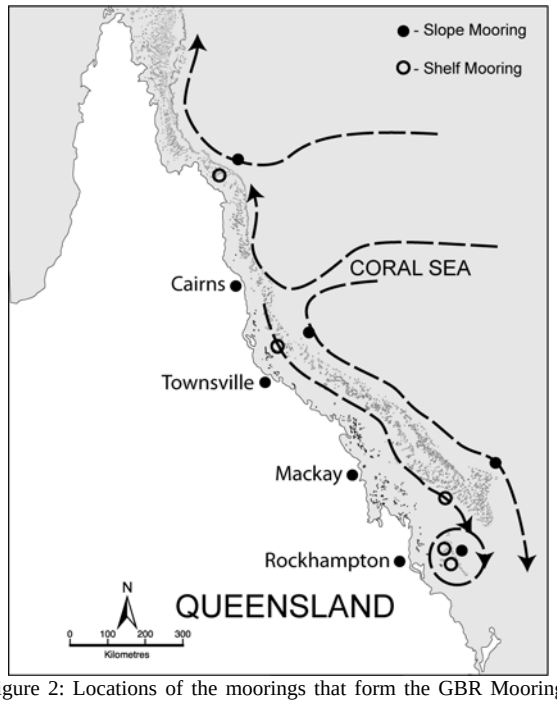

Figure 2: Locations of the moorings that form the GBR Mooring Array, slope moorings are shown as solid markers, shelf moorings as open markers. The major currents are shown as dashed lines based on current surface flows (from Steinberg 2007).

The basic current flows (Steinberg 2007) show the main south equatorial current impinging on the GBR in the Cairns area with one flow going north as the Hiri current and one going south to form the East Australian Current. The southward flow includes components that go down the outside of the reef matrix and those that flow through the larger GBR lagoon. The two flows meet in the southern part of the GBR where a complex series of eddies occurs before the main southward flow becomes coherent.

The pair of moorings off Townsville looks to measure water intruding from the deeper slope onto the shelf or reef matrix so supplying coastal reefs with oceanic water. In this area the reef matrix is open and so water is able to move up and across the shelf. The moorings around Lizard Island are designed to measure the northward current flow outside the reef matrix and the inflow of that water through the ribbon reefs into the reef matrix. The moorings south of Mackay are positioned to measure the southward current flow outside the reef matrix via the deep water mooring and through the main reef lagoon using the inshore mooring.

Around Heron and One Tree Islands three moorings have been deployed, two shelf and one slope, to capture the complex nature of the currents in this area.

The deeper moorings do not have a surface float and so log their data, the shallower shelf moorings will be converted to a surface buoy and will have real-time communications installed. This will allow for summary real-time data to be communicated; the instruments will continue to log the full data set which is retrieved on servicing. The moorings are serviced every six months.

The moorings typically contain a sub-surface float at 10 m, a series of Sea-Bird SBE39 temperature and pressure sensors down the mooring giving surface (10 m), middle and bottom measurements with five minute sampling. These are complimented by two WetLabs WQM's deployed to give upper and lower water column temperature, salinity, turbidity and fluorescence also sampling at five minutes. Currents are measured with a near-bottom mounted Acoustic Doppler Current Profiler (ADCP). For shallow moorings (< 100 m) an RDI 300kHz ADCP is deployed with a sampling of 30 minutes at 25 four-metre 'bins' or intervals, for deeper moorings a Nortek 600kHz ADCP is used with 50 four-metre bins. Some moorings have a Nortek AWAC for waves and currents. At the start and end of each deployment a CTD cast is done with a Sea-Bird SBE19+ to give validation data for the deployed instruments as well as giving start and end profiles.

The final component is the wireless sensor network. Sensor networks will be deployed at seven sites, four island research stations (Heron and One Tree Islands in the southern GBR, Orpheus Island in the central GBR and Lizard Island in the northern GBR) and three reefs (Davies, Rib and Myrmidon Reefs, all in the central GBR). The locations are shown in Fig. 3.

At each site a high speed data link is installed using microwave links or 3G phone technologies. An on-water wireless network is then created using a series of network repeater poles located around the reef and a 900 MHz spread-spectrum radio system.

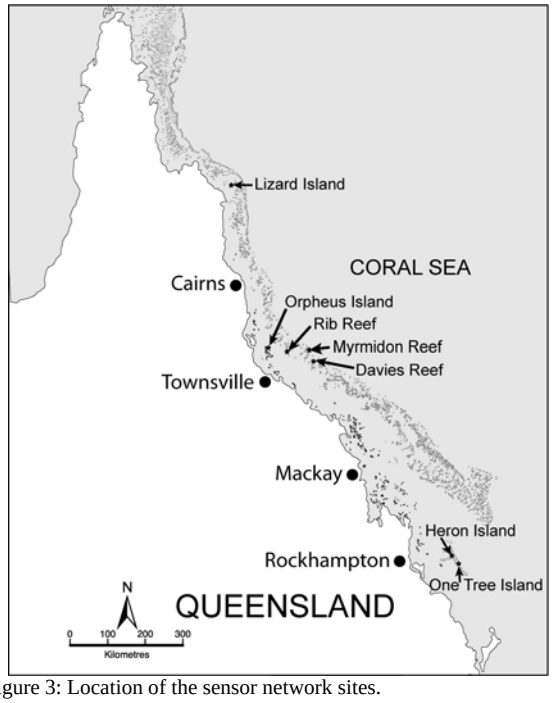

Figure 3: Location of the sensor network sites.

Into this network are located the sensor floats onto which the actual sensors are attached. The floats contain a logger and radio that transmit the data back to the base station and then, via the data link, back to AIMS. The data is relayed in real-time and made available via streaming sensor middleware.

The design allows for areas to be intensively wired up and monitored, much of the equipment is relocatable and can be deployed around individual coral heads and bommies giving real time information about the conditions around the corals themselves. The initial sensors include temperature, salinity and depth but the infrastructure is suitable for other sensors, such as video, partial pressure of CO_2 (pCO_2) and photosynthetically active radiation (PAR). The involvement of the island research stations that form the Tropical Marine Network (TMN) allows for cleaning and maintenance of equipment and the specific deployment of equipment for short-term experiments.

Affiliated with the GBROOS Node is the Australia Coastal Ocean Radar Network (ACORN) facility that is installing WERA based HF coastal radar systems. A system will be based in the southern GBR to give coverage over most of the southern GBR area including the moorings and sensor network sites in the Heron Island area. The coastal radar will therefore provide additional information about waves and surface currents in this area.

All of the data will be made freely available as soon as possible via a dedicated GBROOS data web site or via the IMOS web site (www.imos.org.au).

Results

The project has been operational since mid 2007 with significant deployments already undertaken and the remaining due to be completed by 2009. The first data from most of the components is now being collected and the data management systems are being built to make the data available.

The remote sensing station was commissioned in May 2008 and is sending data to the Bureau of Meteorology for inclusion in their processed products along with some of the vessel based validation data.

The underway systems have been installed on two research vessels with the commercial ferry to be completed by mid-2009. Some initial data has been collected; this is currently being processed and will be available from the final data web site.

The Townsville reference mooring has been deployed in a reduced mode without the surface float and so the data is currently being logged. The station will go to real time in the first half of 2009. The Darwin station is due to be installed in mid-2009 as a real-time buoy with the data available soon after.

The mooring array has been operational since mid 2007 with service/data download visits being performed every six months. The initial data is being analysed and the data management systems are being built so that this data can be made available on the web. There has been a delay in doing this as there is a need to first develop some underlying data standards within the IMOS community and this is taking time.

The initial data from the southern GBR moorings shows periods of distinct stratification of the water column inter-dispersed with mixing events that mix the water down to 40 or more metres. This data is being analysed against the remote sensing and coastal radar data to identify the source and cause of these mixing events and to link these into movements of the currents in this area and events such as upwelling.

Three of the seven sensor network sites have been deployed; these are Heron and One Tree Islands in the southern GBR and Davies Reef in the central GBR. The deployments at One Tree Island consist of three sensor poles, one in each of the smaller lagoons within the main One Tree Island lagoon. The sensor poles have a series of thermistors going from reef flat down into the deeper water of the lagoon. At Heron Island five sensor floats have been deployed around the lagoon each with a surface thermistor, as well the six network relay poles have a single bottom thermistor attached. At Davies Reef the sensors currently deployed include a weather station and a number of sensor floats with surface thermistors. Additional sensors will be added to the existing deployments by mid 2009, these will include depth (pressure) and salinity.

Initial results from One Tree Island Lagoon from the sensor network component for late September 2008 (see Fig. 4) show a series of daily cycles in temperature with increasing temperatures as the days go by and then a series of events that re-set the temperatures. This may be caused by periodic mixing of colder oceanic water into the relatively protected lagoon or by a series of cold-water events pushing colder water into the lagoon. This pattern is also seen at nearby Heron Island indicating that larger scale processes may be at work such as cold water moving onto the reef or through weather events that reduce the surface temperature of the water. A reef-based weather station is due to be installed that may shed some light on the cause of these events. This result shows that there are processes operating at a weekly scale that have the ability to alter water temperatures by four to five degrees in rapid periods of time.

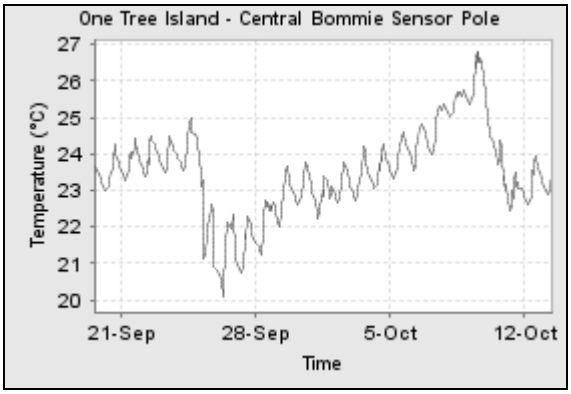

Figure 4. Plot of temperature over time for the bottom (lagoonal) sensor on the sensor pole located in Central Bommie, One Tree Island lagoon, southern Great Barrier Reef for late September 2008.

The project is committing resources to develop data management systems for all of the data being collected. The systems include data archiving of files, quality control of the data, production of metadata (to ISO 19115 standard) and the ingestion of data into a common database schema.

The work includes the development of service based data access routines where access to data and data products (such as Google Earth files, GIS files) is done automatically via user or client submitted URL's. This will allow web sites to be built around the data and for other users to tap into the data using their client of choice, from Matlab, Excel to simple text editors.

For the mooring data the project will help develop a 'toolbox' of routines for processing the raw instrument data as well as an agreed to final file format. With the sensor network data the project is deploying specialised streaming data middle-ware (such as the Data Turbine software) to give users access to real-time data with minimal or no delay.

Discussion

Coral reefs face a range of threats and globally the outlook for coral reef systems is not good. Wilkinson (2004) estimates that some 20% of reefs globally have been effectively destroyed with another 24% under immediate threat and a further 26% under longer term threat. Issues such as climate change are more insidious as the impacts cannot always be dealt with by local management activities and so even reefs that are well managed can be impacted. This disconnect between the impacts that are causing declines in coral reef systems and the local management of the reefs represents a dramatic new turn for the conservation and sustainability of coral reef systems worldwide.

The need is to better understand how each system is exposed to, and impacted upon, the ocean systems that provide much of the water that feeds into the reef and how changes in currents, in upwelling and circulation, impact the long term sustainability of these reefs. Critical to this understanding is the ability to monitor these systems and in particular the interactions with the main oceanic water systems. Observing systems such as GBROOS are designed to help understand and monitor these processes.

GBROOS provides data at range of scales; from synoptic remote sensed imagery of sea surface temperature and ocean colour to underway sampling that gives surface water information across regular transects to the fixed mooring sites that provide long term detailed profile data at key locations. The sensor networks provide re-locatable fine scale information at the level of individual coral heads. Being able to link data from the scale of tens of kilometres down to tens of centimetres is unique and hopefully will provide the connectivity information required to fully understand the impact of large scale oceanic events on the microclimate and biological responses of individual corals.

Future work will look at the linkages between the various observing components and in particular to investigate the linkage between large scale ocean processes and small scale within reef processes and subsequent biological responses. A series of experiments are planned for 2009/10 at Heron and One Tree Islands to develop and validate models that better express the linkage between processes operating at differing scales.

In the northern sites the work will focus on tying biological processes, such as spawning, into the physical parameters and to again develop models of behaviour and responses that are driven or impacted by physical water conditions. The aim is to better understand these processes and the potential and real impacts of change on the fundamental biological systems.

Under the governance model of the project all data will be made freely available as soon as possible with much of it being in real-time. The infrastructure of moorings, communications systems, data management and logistical support will be made available to the marine science community via a competitive bidding system managed by IMOS. This means that the GBROOS data and infrastructure are open to all and that as such GBROOS belongs to the science community not to a few agencies.

GBROOS looks to provide the data, through a range of real-time and delayed mode data streams that will facilitate the development of new models and understandings of how oceanic processes impact and drive coral reef systems and how changes in these large-scale systems are related to smaller scale events such as the bleaching of an individual coral. Being able to measure the entire thermal history of a coral head will give much better understanding as to why some corals bleach and others don't.

Observing systems will not replace divers or field work but as they are able to provide real-time, long-term and *in-situ* data they are an important part of understanding how these systems function and how the biological responses observed in the field link to the fundamental oceanic forces that drive reef systems. GBROOS is a first step in a systematic attempt to provide the information required to support modelling and visualisation work and to support a range of fundamental and applied science.

Acknowledgement

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Ecological forecasting for coral reef ecosystems

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Abstract. Assessment of coral reef ecosystems implies the acquisition of precision data and observations appropriate for answering questions about the response of multiple organisms to physical and other environmental stimuli. At the National Oceanic and Atmospheric Administration's Atlantic Oceanographic and Meteorological Laboratory, we model marine organismal response to the environment in terms of a Stimulus/Response Index (S/RI). S/RI is computed using an approach called heuristic programming, from parameters bounded in subjective terms, which are defined numerically by comparing historical data with expert opinion, so as to match research and our understanding of the process in question. The modeled organismal response is called an ecological forecast, or ecoforecast, and relative possibility and intensity of the response is reflected in a rising S/RI. We have had success to date in modeling coral bleaching response to high sea temperatures plus high irradiance and other parameters. The approach requires, a) highly robust instrumentation (in situ, satellite, or other) deployed for long periods and producing high quality data in near real-time, b) a basic understanding of the process, behavior and/or physiology being modeled, and, c) a knowledge of approximate threshold levels for single or synergistically acting environmental parameters that elicit the phenomenon in question.

Key words: coral reef · artificial intelligence · bleaching/stress model · data integration · NOAA

Introduction

As stewards of the marine environment in US territorial waters, the National Oceanic and Atmospheric Administration (NOAA) must continually strive to understand how marine ecosystems respond to environmental and anthropogenic change so as to protect its fisheries resources, shorelines, and areas of high biodiversity and intrinsic beauty, such as the many square miles of coral reefs, sea grass and kelp meadows, and other ecosystems. Because of the accumulating stresses through global warming, toxic and eutrophying effluent, and opportunistic and introduced invasive species, detecting early change in the environment becomes not just desirable but critical to the well-being of ecosystems and humans alike, especially when remedial action can only be taken less expensively at an early stage. At the Atlantic Oceanographic and Meteorological Laboratory (AOML), the Integrated Coral Observing Network (ICON) has since 1998 (Hendee et al. 1998) improved upon a series of artificial intelligence techniques to produce near real-time data-driven models of how organisms or events are influenced by meteorological and oceanographic stimuli acting singly and synergistically. These models, when validated and used to provide decision support for Marine Protected Area (MPA) managers, or to add to researchers' knowledge of stimulus by the environment (as well as response by organisms or ecosystems), are called ecological forecasts or "ecoforecasts," and are more formally defined as predicting "the impacts of physical, chemical, biological, and human-induced change on ecosystems and their components" (CENR 2001; Brandt et al. 2006).

Thus, the ICON program has developed a numerical measure of the response by organisms and ecosystems to these impacts called a Stimulus/Response Index, or S/RI. The S/RI can also serve double-duty by informing station maintainers and AOML researchers of any drifting or otherwise errant environmental data when those values are found to be outside acceptable ranges as defined by ecoforecast models.

Measuring the Environment

Sensors in the marine environment have to be routinely maintained and calibrated to ensure quality data or the whole exercise of producing ecoforecasts would be fruitless. Stations we at AOML produce for deployment are called Coral Reef Early Warning System (CREWS) stations, named after the early ecoforecasting software developed by Hendee et al. (1998), and later improved (Hendee et al. 2006; 2007).

CREWS stations have been deployed near Lee Stocking Island, Bahamas; Salt River Bay, St. Croix, US Virgin Islands (USVI); La Parguera, Puerto Rico; and Discovery Bay, Jamaica; with new stations over the next two years currently being planned for Brewer's Bay, St. Thomas, USVI; Little Cayman, Cayman Islands; Managaha Bay, Saipan, Commonwealth of the Northern Marianna Islands; and Kenting National Park, Taiwan. These stations produce meteorological and oceanographic data: wind speed and direction, barometric pressure, precipitation, light (above and below water), sea temperature, salinity and tide height. Research specific instruments have also been deployed: partial pressure of CO_2 and pulse amplitude modulating (PAM) fluorometry. Realizing the goal of maintained and calibrated instruments requires participation of field scientists and technicians, who also provide necessary support by validating ecoforecasts produced from AOML. The instrumental architecture (data acquisition and transmission) for CREWS stations has been elaborated elsewhere (Jankulak et al. in press).

The ICON program also produces ecoforecasts from data received from other in situ networks: the SEAKEYS Network of stations in the Florida Keys (Ogden et al. 1994); NOAA AOML's South Florida Ecosystem Research and Monitoring Program stations also in the Keys (SFP 2008); NOAA's National Marine Fisheries Service (NMFS) network of buoys maintained by the Coral Reef Ecosystem Division (Hawaii) in the Pacific; and the Australian Institute of Marine Science's Weather Network of eight stations on the Great Barrier Reef (Berkelmans et al. 2002) Most of these stations are well maintained, but some are so remote it is difficult to do this in a timely way.

On the assumption that sea-surface data derived from satellites are relatively good, considering their well studied and proven algorithms, data are also received from latitude/longitude pairs representing target sites for multi-parameter data collection, and called "virtual stations" (Hendee et al. 2008). Satellite data have been collected from 111 virtual and in situ station sites to date, from all three major oceans, with more planned for the future. Ecoforecasts produced from these data are always compared with in situ stations when there are some nearby, but that is not always the case. In all cases, field validation of ecoforecasts is necessary to improve or correct them.

Finally, there is one other source of data for ecoforecasting at Molasses Reef and nearby sites in the Florida Keys National Marine Sanctuary (FKNMS): Wellen Radar (Shay et al. 2002). This source of data continuously tracks surface currents, which are useful in onshore flux (Gramer et al. 2008) and larval drift ecoforecasts (L. Gramer, unpublished).

Information Architecture

Fig. 1 displays the flow of satellite and other data from a site, through a data clearing hub, and finally back to AOML for posting to a Web page which displays the most recently transmitted Web data, as well as any ecoforecasts operational for the day.

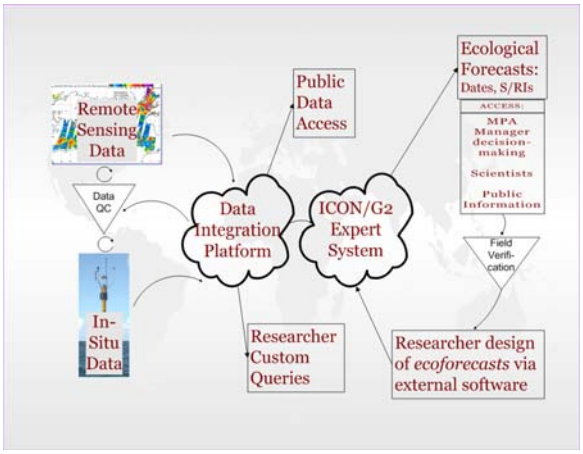

Figure 1. ICON information architecture.

Reducing Environmental Complexity

The CREWS approach to ecoforecasting seeks to reduce the extreme complexity of modeling the contributions of multiple environmental factors on eliciting behavioral and ecosystem response. Organisms respond rarely to one environmental factor; rather, they respond to a complex and often synergistic combination of factors, for instance temperature and light. One of the most complex concepts to study and understand is how the physiology of an organism that responds to, say, sea temperature and light, responds differently to a range of values for each parameter, but in combination. For instance, consider reaction to a high sea temperature and low irradiance value vs. a high sea temperature and a high irradiance value. Ideally, one would construct an index of response to an arbitrarily fine series of gradations of each of these parameters (e.g. for sea temperature, 29.01, 29.08, 29.11, etc.) in various combinations. Obviously, the number of combinations and permutations for more than one parameter quickly becomes very large. What we have done is to adopt a heuristic modeling approach. In computer science, a heuristic is "a technique designed to solve a problem that ignores whether the solution can be proven to be correct, but which usually produces a good solution or solves a simpler problem that contains or intersects with the solution of the more complex problem" (Wikipedia entry: "Heuristic" 2008). Utilizing an expert system tool called G2 (Gensym, Inc.) we use subjective terms to

describe data levels and times of day, then use those terms in "production rules" (basically, if/then constructs) to model cause and effect scenarios. To do this, we first subdivide the continua of each parameter into discrete subjective levels ("bins"), based on what historical data show are minima and maxima, and according to what researchers and users of data consider appropriate. For instance, in the open ocean, salinity readings of 34-36 psu would be considered "average" or normal, while a reading of 23 psu would be considered "unbelievably low." Table 1 lists the subjective terms we use for levels of many sensor data, along with the abbreviations utilized within the software.

Table 1.CREWS/G2 subjective data ranges.

Abbrev	Description
ul	unbelievably low
dl	drastically low
vl	very low
lo	low
sl	somewhat low
av	average
sh	somewhat high
hi	high
vh	very high
dh	drastically high
uh	unbelievably high

Table 2.
CREWS/G2 subjective daily periods.

Abbrev	Period	GMT Time	Local (@5 hrs)
(Basic Periods)			
midn	midnight	0300 - 0600	2200 - 0100
pdaw	pre-dawn	0600 - 0900	0100 - 0400
dawn	dawn	0900 - 1200	0400 - 0700
morn	morning	1200 - 1500	0700 - 1000
midd	mid-day	1500 - 1800	1000 - 1300
psun	pre-sunset	1800 - 2100	1300 - 1600
suns	sunset	2100 - 2400	1600 - 1900
even	evening	0000 - 0300	1900 - 2200
(Large Groupings)			
all	all-day	0300 - 0300	2200 - 2200
dayl	daylight-hours	0900 - 2400	0400 - 1900
nite	night-hours	0000 - 0900	1900 - 0400
dayb	dawn-morning	0900 - 1500	0400 - 1000
aftn	afternoon	1800 - 2400	1300 - 1900

Naturally, data change with time, and so does the response of an ecosystem or an organism. Table 2 shows various subjective assignments for periods of the day, following the same sort of logic as for data ranges. An additional advantage of this approach is that organisms most often are active at periods we can more easily identify with. For instance, a nocturnal animal is active during "night-hours," a diurnal animal during "daylight-hours," and a crepuscular animal during "dawn" or "sunset," and so on. This helps in the descriptive phrasing of our final ecoforecast product.

Calculating the Stimulus/Response Index

The final pre-processing of data before their utilization in an ecoforecast model is the assignment of "points" to reflect the duration of the received value at the subject level and for the subjective time of day. The points accrue to a relatively simple indexing method which assigns one point to each hour that the parameter remains in one of the subjective daily periods (Table 2), multiplied by the subjective data range (Table 1), with the latter being the key determinate of the degree of stimulus or "stress." Thus, values near "average" values are treated with a multiplier of 1, while the "very low" and "very high" designations use 2, and the "drastic" levels a multiplier of 2.5.

A decision table is a complex if/then statement which allows the knowledge engineer (a person who constructs expert systems) to weigh different input levels in the decision making process. Fig. 2 shows the use of a decision table to explain our approach in an example of a coral bleaching model, and shows how the stimulus/response index is formulated.

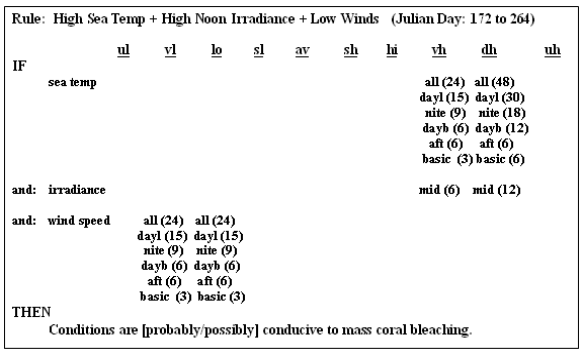

Figure 2. A decision table for coral bleaching, modeling input of sea temperature, irradiance and low winds as parameters of stress. Refer to Tables 1 and 2 as reference to the use of the abbreviations (e.g. ul, lo, dayl, basic, etc.).

To facilitate understanding, the figure may first be interpreted in a simple fashion thus: if sea temperature is high, and irradiance is high, and wind speed is low, then conditions are likely conducive to mass coral bleaching. However, the use of our point (index) system is to assign points for the duration and time of day. Therefore, the most extreme limits of stimulus (or stress) are assigned a point level of 2X or even 2.5X the number of hours of duration. So, though a 24 hour duration of "high" sea temperature would accumulate 24 points, one that is "very-high" would accumulate 48 points, and a "drastically-high" duration rates 60 points. This point system is

assigned to reflect the rarity of the "drastic" levels for each particular parameter. In the end calculation, the stimulus (or, in this case, stress) points are added up for each of the conducive parameters (e.g. sea temperature, light, winds) to arrive at a Stimulus/Response Index (S/RI).

In summary, utilizing this approach, the data process, from acquisition at the sensor to preparation of the data for a heuristic model challenge, is:

- Data are received as hourly averages from the collection site and "tagged" as to the appropriate parameter (e.g. "sea-temp").
- Data are categorized as to one of the subjective levels, as in Table 1.
- Depending upon what time of day the data are collected, they are further categorized according to Table 2 into one of the Basic Periods (three hours in length each).
- If the subjectively categorized data continues in that range beyond its originally assigned three hour period, it is further re-categorized according to the next larger period as appropriate, up to possibly "all-day."
- If the data persist at a level of stimulus (or stress) qualitatively beyond the basic threshold, they are assigned a multiplier great than unity to indicate numerically the greater stress.
- Indices from all the stimuli are added to come up with a final S/RI value, which indicates the relative intensity of the modeled response, and the relative likelihood of any response of the modeled type, to the environment at the time it is monitored.

Ecological Forecasting

Once the number of points is assigned in a heuristic model, an assessment is made as to whether those points reach a threshold level for reporting as an ecoforecast, either via an email message or Web posting to decision makers, researchers, field technicians or the knowledge engineers. Once these ecoforecasts are received, then the desirable outcome is for the field personnel (technicians maintaining a station, researchers, interested public, etc.) to give timely feedback as to whether the ecoforecast was correct as to the outcome. If it is was not correct, feedback to the knowledge engineers allows further fine-tuning of the model until, through time, it becomes a reliable ecoforecast for decision makers.

There are now many types of ecoforecasts produced as part of the ICON project, accessible via the project's Web presence (ICON 2008) which provides both hourly data from various sources at coral reef areas around the world, and ecological forecasts produced from those data. The structure of the ecoforecasts reflects one of the hallmarks of a true expert system, namely, that results are explained (i.e. "because") so that those who use the output can follow the reasoning and agree or not, and thus give feedback to the knowledge engineer so that proper refinement of the rules can take place. Figs. 3 and 4 show this approach and illustrate a current experimental coral bleaching ecoforecast being produced at Salt River Bay, St. Croix in the U.S. Virgin Islands.

Results

Early in the history of the CREWS software, the ecoforecasts were called "alerts" and were produced for the SEAKEYS Network (e.g. Hendee et al. 2001), the AIMS stations on the Great Barrier Reef (Berkelmans et al. 2002; Hendee and Berkelmans 2003), and in St. Croix (Manzello et al. 2006). With the current addition of more complex coral bleaching models reproduced at over one hundred sites around the world, as well as new types of ecoforecasts, it is now important to gain more feedback from researchers and collaborators in the field to validate and utilize them for decision support for MPA managers, and to help researchers understand how coral reef ecosystems respond to a changing climate.

```
Ecoforecast Rule #4: Coral-Bleaching-Itlw
  Description: Mass coral bleaching (high in-situ sea temperature + high light + low wind)
  This rule will produce a forecast *IF* . . .
      'Photosynthetically Active Radiation At Ocean Surface (parsurf)' has any of these values:
           High Very-High Drastic-High
      (Within any of these 3-hour or longer periods: Mid-Day Pre-Sunset Daylight-Hours Afternoon All-Day)
  AND . . .
      'Hourly Average Wind Speed (windsp)' has any of these values:
           Drastic-Low Very-Low Low
      (Within any of these 3-hour or longer periods: Morning Mid-Day Pre-Sunset Daylight-Hours Dawn-Morning Afternoon All-Day)
  AND . . .
      'Depth-Averaged Sea Temperature (seandbc)' has any of these values:
           High Very-High Drastic-High
      (Either throughout the day, or during any 3-hour or longer subperiod thereof.)
```

Figure 3. Representation of the decision reasoning on the Web site for a coral bleaching ecoforecast, considering high sea temperature, high irradiance and low winds.

Ecological forecast: 'Mass bleaching', for 2005-Sept-28, Salt River, St. Croix, US Virgin Islands
Model rule: 'Mass coral bleaching (high in-situ sea temp + high light + low wind)'
Stimulus/Response Index (S/RI) = 9, because:
Photosynthetically active radiation at ocean surface (PARsurf) was drastic HIGH (1827) during period Mid-Day
Hourly averaged wind speed (Windsp) was LOW (1.8) during period Morning
Sea temp at 1 m (SeaT1m) was HIGH (30.7) during period Pre-Sunset

Figure 4. Coral bleaching ecoforecast output, considering high sea temperature, high irradiance and low winds.

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Autonomous Underwater Vehicles resurvey Bonaire: a new tool for coral reef management

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Abstract. Bonaire's reefs are among the most pristine in the Caribbean. Creation of the Bonaire National Marine Park in 1979 set an important precedent for Marine Protected Areas. Van Duyl (1985) published an underwater atlas of the bottom type and benthic community to a depth of 10 m, but there have been few surveys of the deeper (>65 m) reef. In 2008, Bonaire's reefs were remapped using three Autonomous Underwater Vehicles (AUVs), Nitrox, and Trimix SCUBA. The AUVs carried high frequency side scan and multibeam sonar, cameras, and water column sensors (CTD, pH, dissolved oxygen). Divers ground-truthed AUV data on bottom-type and biota present. AUVs were used because they can survey deeper, cover more area over shorter time periods, and simultaneously collect multiple datasets compared to surveys by ship or SCUBA. A preliminary examination of expedition data shows higher diversity (H') and coral percent cover as one proceeds from south to north. The AUVs successfully mapped the bathymetry, and collected data on bottom type, fishes, coral cover and community type at locations along the entire leeward coastline. Our work shows the potential for AUVs working in conjunction with divers to provide a new tool for reef assessment at the landscape level.

Key words: Autonomous Underwater Vehicle (AUV), Bonaire, multibeam sonar, side scan sonar, reef atlas

Introduction

Bonaire (Fig. 1A), Netherlands Antilles, is arguably among the most pristine coral reef environment in the Caribbean (Steneck and McClanahan 2004). Percent coral cover is the highest and percent algal cover the lowest compared to other Caribbean reefs (Kramer 2003) and thus it represents a baseline *sensu* Jackson (2001), with herbivory implicated in its health (den Haan et al. 2008). Bonaire's economy depends mainly on tourism with almost 30,000 SCUBA divers entering its waters in 2006 (DEZA 2006). Bonaire's reefs are among the best protected in the Caribbean with no collections of any kind allowed, with the exception of a hook and line fishery. The Bonaire Marine Park Authority oversees the administration and protection of this unique underwater resource with help from STINAPA (Stichting Nationale Parken) Bonaire, a not-for-profit foundation. Although the shallow environment near Bonaire has been extensively visited by tourists and scientists, little to no survey work has been conducted on the deeper reef (60-100 m) on into deeper water (100-300 m) (R de Leon, STINAPA, pers comm.). A recent meeting of the International Coral Reef Initiative, an effort of the United Nations Environmental Program, identified mapping of the reefs of Bonaire as a top priority (ICRI 2005).

Bonaire's reefs were extensively mapped in the early 1980s by van Duyl (1985). The resulting atlas (Fig. 1B) was compiled from high-resolution aerial photography, ground-truthed by extensive SCUBA diving. It provides a snapshot of reef cover, substrate type, and geomorphology around the time that full protection for Bonaire's waters was enacted. Long-term monitoring of Bonaire and Curaçao's reefs has been ongoing at specific locations since the 1970s (Bak 1977). Bak et al. (2005) analyzed a 30-year time series of permanent quadrats and found that while there has been some degradation of reefs in Bonaire in shallow water, corals deeper on the reef have remained at similar population levels over several decades. However, a large scale synoptic mapping effort of the scale of van Duyl (1985) has not been attempted.

AUVs are free-swimming robots that act as taxicabs for sensors. They can cover many km per day in close proximity to the seabed, providing enhanced resolution for multibeam (bathymetric) and side scan imaging sonars. AUVs can simultaneously acquire video imagery and water quality data (Patterson and Sias 1998; Hayes et al. 2007). They can also use advanced pattern recognition techniques to classify water column targets in the nekton seen on side scan or multibeam sonar down to the species level (Patterson et al. 2007). Diver video survey strategies (e.g. Aronson et al. 1994) can thus be complemented by AUVs, as AUVs can dive deeper and longer than humans, and gather more data types concurrently.

The goals of our expedition were to (1) test the efficacy of using AUV technology to conduct

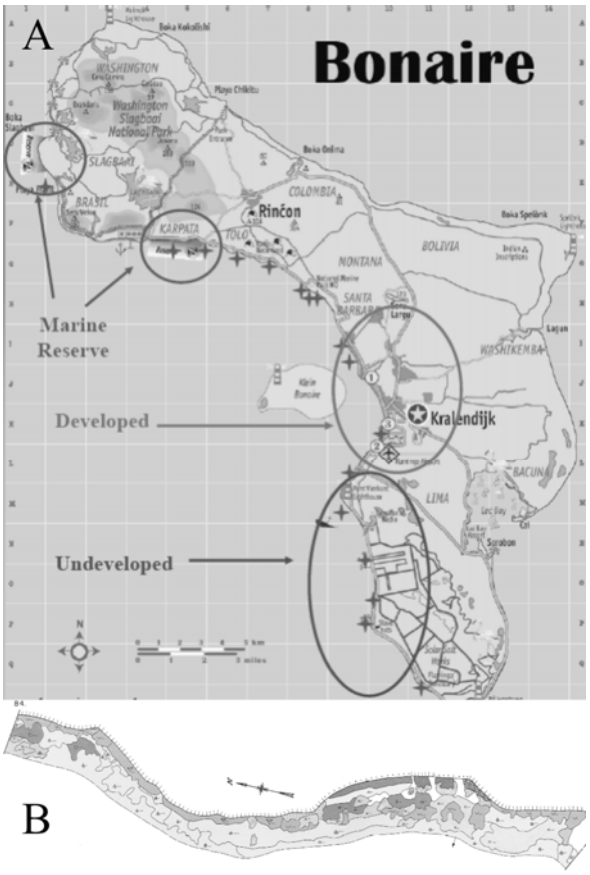

Figure 1: A. Areas targeted for diver and AUV surveys per recommendations of Bonaire government officials. Crosses represent locations where shore-perpendicular diver transects and AUV operations occurred. B. Example of a map created by van Duyl (1985). Eight community types from a set of 22 defined for Bonaire are represented in this view of the coast near Slagbaai.

bathymetric and side scan sonar surveys and video mapping in a coral reef setting, (2) concurrently characterize the physical and chemical oceanography, and (3) ground-truth the AUV data using diver surveys both shallow (Nitrox) and deep (Trimix). The long-term goal is to compare our maps with the previous survey of van Duyl (1985) to discern trends in the status of Bonaire's reefs.

Materials and Methods

Three AUVs (Fig. 2) were deployed from small boats or from shore at or near the locations shown (Fig. 1A). AUV payloads and characteristics are shown in Table 1. AUVs were operated in terrain-following and depth-holding mode. Each AUV was operated by a team of two to three people. Each of the Gavia AUVs logged about 120 dives ranging in length from 10 min to 2 hrs and depths from 2 to 220 m. The VIMS Fetch1 AUV completed 100 dives of 3 to 20 min duration over depths of 2 to 70 m.

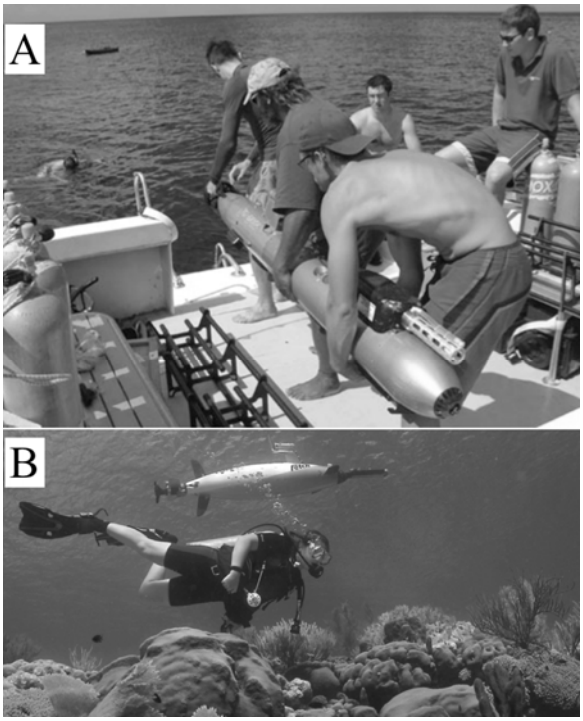

Figure 2: AUV technology used to map Bonaire's reefs. A. Two Gavia-class AUVs were deployed from boats and the shore on the leeward side of Bonaire. B. The VIMS Fetch1 AUV above a diver.

Diver video surveys were conducted using Nitrox SCUBA in a shore-perpendicular direction to a depth of 25 m, to allow ground-truthing of shallow-water AUV surveys. One diver towed a surface float containing a GPS (Garmin eTrex) set to logging mode. A second diver swam directly behind the first, and recorded video from an altitude of 2 m using a Canon Powershot SD950 IS digital camera in an underwater housing, on video setting with a resolution of 1024 x 768 pixels, shooting fifteen frames per second with no additional lighting. Trimix dive operations were conducted at a subset of the 17 areas noted in Fig. 1A, and gave us valuable phototransect data at depths as great as 81 m. Phototransects were also conducted at 40, 30, and 20 m. Video imagery was collected at several levels of resolution (Figs. 3 & 4). Video data were analyzed using Coral Point Count with Excel extensions (Kohler and Gill 2006), using 200 random points per video frame (Fig. 3C).

Results

The expedition successfully collected sonar, video, water quality, and still images over a substantial depth range. These data were used to generate percent cover, Shannon index (H'), and community composition, for comparison of deep vs. shallow reefs and sites along the coast. A full analysis comparing our data to van Duyl (1985) will be presented elsewhere. However, preliminary analysis of the diver

Table 1: AUV parameters. Abbreviations: VIMS = Virginia Institute of Marine Science; UBC = University of British Columbia; SLA = sealed lead acid; MSTL = Marine Sonic Technology Ltd.; CTD = Conductivity, Temperature, Depth; ADCP = Acoustic Doppler Current Profiler; DVL = Doppler Velocity Log; INS = Inertial Navigation System.

AUV / **Param.**	***VIMS Fetch1***	***UBC Gavia***	***Hafmynd Gavia***
Length	2.3 m	2.4 m	2.7 m
Weight	91 kg	55 kg	78 kg
Swim time	4 hrs	6 hrs	6 hrs
Batteries	SLA	Li-ion	Li-ion
Depth	100 m	500 m	250 m
Side scan sonar	MSTL 600 kHz	Imagenex 220/990 kHz	MSTL 900/1800 kHz
Multibeam sonar	None	None	GeoSwath Plus 500 kHz
Other payloads	CTD, pH, O_2, analog video	CTD, ADCP/ DVL, digital camera + strobe, fluorescence	Kearfott INS, digital camera + strobe, sound velocity

video data shows higher diversity (Shannon index H') and coral cover proceeding from south to north (Tukey multiple comparison, $p < 0.005$), and that deep reefs at all three sites are more diverse than their shallow reef counterparts. Percent cover is also higher with depth for these three sites (Table 2).

Landscape-level patterns can be discerned from photomosaics constructed from AUV datasets (Fig. 4). The fore-reef slope image shown was created from data gathered during a terrain-following dive by Fetch1. The resolution is sufficient for identification of coral genera, percent cover, and community type *sensu* van Duyl (1985). Individual camera frames were automatically adjusted for illumination and color balance. The mosaic was created in ptMac (Kerkus) using four to six control points per frame. Coral bleaching and coral mortality, overgrowth of corals by nuisance species like the tunicate *Trididemnum solidum*, and cyanobacterial tufts are detectable in AUV videos from Bonaire.

The deeper reef below 60 m, and the surrounding sand and rubble bottom in even deeper water, are virtually unexplored. The photo imagery gathered from these deeper dives will be the subject of a future publication. The expedition proved that AUVs gathering video provide a new tool for rapid reef assessment down into the twilight zone, where surface irradiance is less than 1% of that at the surface.

High frequency sonar data provided interesting insights into the geomorphology, reef structure, and

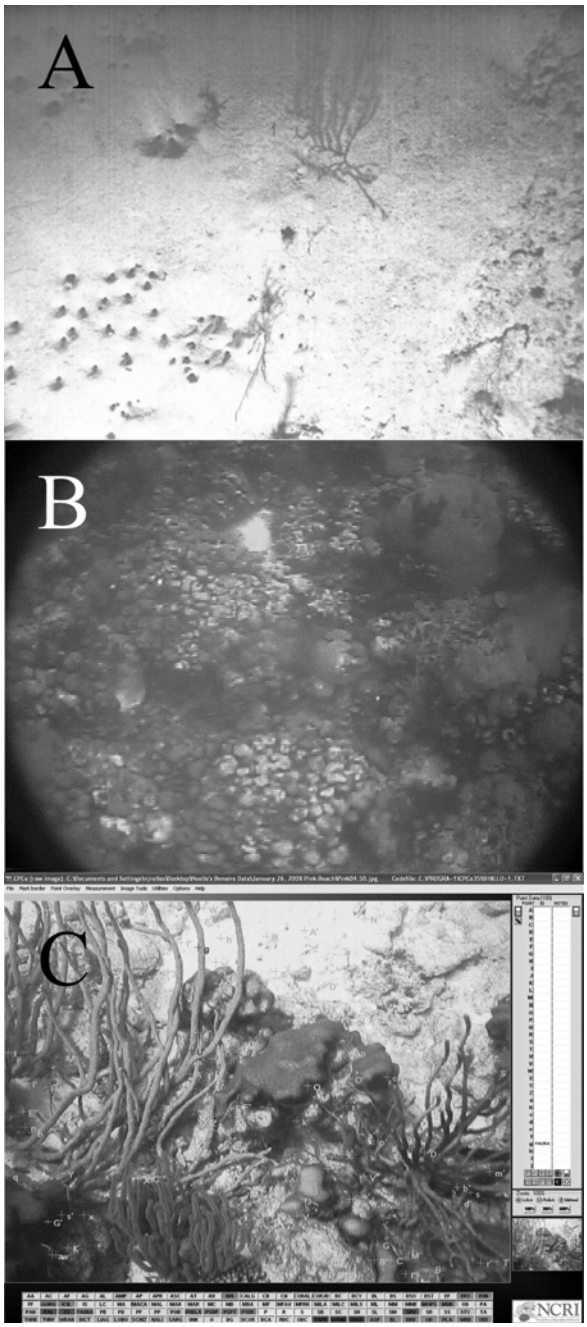

Figure 3: Examples of video imagery collected during the expedition. A. Twilight zone (65 - 200 m) of the reef was imaged by the Gavia AUV. This image is from 147 m. B. Shallow-water landscapes were imaged by the VIMS Fetch1, flying at an altitude of four m. C. Shore perpendicular and parallel transects were conducted by Nitrox and Trimix SCUBA, and were analyzed using Coral Point Count with Excel extensions, shown here in a screenshot (Kohler and Gill 2006).

even habitat utilization by fishes (Fig. 5). Relict reefs, spur and groove formation, double reefs, and bottom type changes can be discerned. Sonar swath widths are much larger than those obtained using video and thus allow the rapid survey and mapping of much larger areas. For example, a 400 m x 400 m area was

completely surveyed at cm level resolution during a single dive by Gavia, using the GeoSwath Plus multibeam module. One of the key features of the GeoSwath Plus is that it collects simultaneous true digital side scan data. The side scan resolution of the 500 kHz system discerns structure down around 3-10 cm depending on survey speed, giving highly detailed images of the seafloor, corals and even fishes in the water column. Using backscatter data, an expert could discern bottom type over the survey area, which was then verified by diver ground-truthing.

Table 2: Mean coral cover and Shannon index (H') at 3 sites surveyed via SCUBA. Sites A, B, C are undeveloped, developed, and marine reserve, respectively. Percent cover (mean) was arc sine transformed for t-test of depth effect, and back-transformed for mean shown above. N is number of video frames.

Site, Latitude Longitude, Depth	N	H'	Coral (%)	Sig. depth?
A, 12° 3.8634' N 68° 16.9236' W, < 5 m	103	0.04	0.5	H', % p = .05
A, 12° 3.8634' N 68° 16.9236' W, > 5 m	70	0.82	13.4	
B, 12° 8.2884' N 68° 16.6050' W, < 5 m	30	0.42	5.5	H', % p = .05
B, 12° 8.2884' N 68° 16.6050' W, > 5 m	26	1.23	23.9	
C, 12° 12.6265' N 68° 19.2892' W, < 5 m	106	1.08	18.4	H', % p = .05
C, 12° 12.6265' N 68° 19.2892' W, > 5 m	28	1.48	47.0	

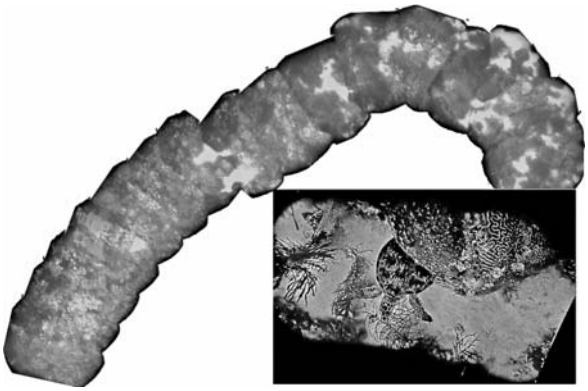

Figure 4: Seascape mosaics can be gathered in a cost-effective manner by AUV technology. VIMS Fetch1 AUV mosaic of fore-reef slope in marine reserve of Bonaire, deeper reef at lower left. Width of frame is 6 m. *Inset:* VIMS Fetch1 AUV image of turtle hiding under brain coral in FL Keys. Flying at lower altitudes allows identification to species level at the cost of reduced swath width (1.5 m), and strong possibility of collision with the bottom.

Discussion

AUV technology can reduce the cost per datum for seafloor mapping, by reducing or even eliminating the need for a ship, which can cost many thousands of dollars per day. AUV surveys also have the potential

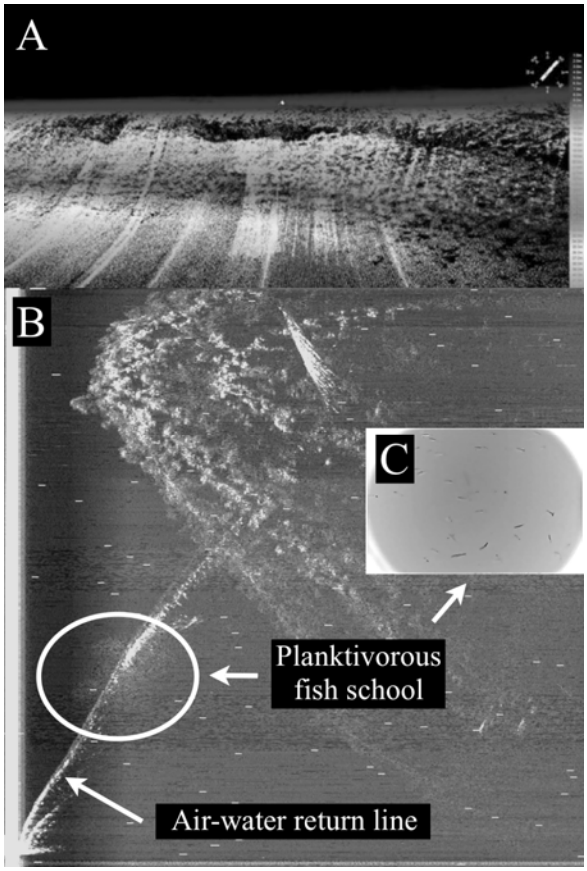

Figure 5: Sonar imagery of Bonaire´s reefs. A. GeoSwath Plus multibeam sonar view of reef, reef slope, and sand channel leading to Klein Bonaire, north of Kralendijk, perspective view facing east, collected by the Hafmynd Gavia AUV. B. MSTL side scan sonar image of fore reef, and water column biota, collected by VIMS Fetch1 AUV. Bright line is sonar return from the air-water interface, and shows AUV turning to begin depth holding. During the dive, Fetch1 passed through a school of planktivorous fishes (*Clepticus parrae*, *Chromis* spp.), verified by imagery from the onboard video camera (Inset C).

to allow rapid mapping of coral reefs at the landscape level complementing aerial or satellite remote sensing (cf. Andréfouët et al. 2003). When combined with diver ground-truthing, AUV surveys provide a synoptic view of coral reefs at scales from cm to 10s of km, and include depth ranges not easily attained by diving.

The high capital cost of AUV acquisition (US $80,000-600,000) depending on manufacturer and payloads) needs to be weighed relative to the 15 year working lifetime per vehicle currently used by insurers (AC Trembanis, Univ. Del., pers. comm.). AUV reliability for this emerging technology is also a concern with new risk assessment methods for operators under development (Griffiths and Trembanis 2007). Use of AUV mission control software that follows a strict state-machine architecture, used on interplanetary spacecraft where human intervention is not feasible, increases

reliability, and was used on the Fetch1 AUV (Patterson 1998; Patterson and Sias 1999).

The seafloor survey rate of an AUV (32,400 m^2/hr) is about 12x that of the divers, based on swath width (6 m for AUV vs. 1.5 m for diver) and swimming speed (1.5 m/s for AUV vs. 0.5 m/s for diver). The AUVs also gathered two kinds of sonar data and water column data concurrently. Using identical personnel and boat costs, straight line amortizations for one $500,000 AUV (15 years) and $4,000 dive gear (4 years) for two people, the above coverage rate, and assuming the AUV gathers five sets of equally spaced data (two types sonar and water quality, video) vs. video only for the diver, the ratio of cost per datum acquired by AUV compared to a dive team is 0.56. Presently, diver surveys using photo-quadrats and handheld video remain the best choice for detailed survey work where identification to the species level is required. Soon AUVs will be able to terrain-follow at altitudes of 1-2 m required for such work (A Steingrimsson, Hafmynd ehf, pers. comm.).

AUV surveys could pinpoint nuisance species outbreaks so that divers could more efficiently remove them. Diver removal of a tunicate has recently been proposed for Bonaire's reefs (McGrath and Peachey 2008). AUVs could provide quick landscape level resurveys after storm disturbance or coral bleaching events. AUVs are emerging as the tool of choice for bathymetric mapping, assessing fish utilization on reefs, and conducting video surveys of the twilight zone. Their use promises to make the interval between valuable snapshots like that taken by van Duyl (1985) and our survey much shorter in the future. AUV surveys can thus help mitigate against shifting baselines by providing timely, cost-effective data acquisition.

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The instrumental architecture of a Coral Reef Early Warning System (CREWS) station

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Abstract. The Integrated Coral Observing Network (ICON) program has constructed and installed a series of Coral Reef Early Warning System (CREWS) stations which provide a wealth of high-quality meteorological and oceanographic data in near real-time. CREWS stations date back to 2001 with the deployment of an early buoy-type design in the Bahamas. Beginning in 2002, the program shifted to a pylon-type design which was re-engineered in 2005, resulting in the modern CREWS stations found in the Bahamas, Puerto Rico, the US Virgin Islands and Jamaica. The CREWS instrumentation architecture described herein has evolved over time into a robust package that, combined with a regimen of regular instrument cleaning and recalibration, has yielded a continuous, long-term, high-quality dataset from these harsh marine environments.

Key words: coral, CREWS, monitoring, instruments, temperature, salinity, light, wind, pylon

Introduction

At the National Oceanic and Atmospheric Administration's (NOAA) Atlantic Oceanographic and Meteorological Laboratory (AOML), the Integrated Coral Observing Network (ICON) program has evolved to provide both near real-time marine ecological forecasts ("ecoforecasts" Brandt et al. 2006; CENR 2001) and hourly checks on the status of the sensors it monitors, whether in situ, satellite or other (e.g., Wellen Radar; Shay et al. 2002). Coral Reef Early Warning System (CREWS) stations are a type of in situ station developed by the ICON program at AOML and so-called because of expert system software originally developed by Hendee et al. (1998), and improved by colleagues (Hendee et al. 2007), to monitor data from the SEAKEYS Network in the Florida Keys (Ogden et al. 1994). Newly engineered stations were developed to begin an expansion into U.S. coral reef areas, in response to recommendations by the U.S. Coral Reef Task Force (unpublished memoranda) and United States Executive Order 13089 (Anonymous 1998), and acquired the nickname "CREWS Stations." CREWS stations typically contain all the usual meteorological and oceanographic instruments, as well as instruments of special interest to specific research projects. The CREWS Network is but one in situ network now monitored by the ICON Program.

The present report expands upon Hendee et al. (2006), in outlining the instrumental architecture for CREWS stations.

Materials and Methods

Surface Instrumentation

Surface instruments on the station pylon (Fig. 1) measure light, wind, temperature, barometric pressure, humidity and precipitation. Most measurements are made redundantly by multiple instruments. Instruments which must be isolated from equipment that could block light or wind are mounted away from the station on aluminum masts. Other surface equipment includes five solar panels, a transmitter antenna, a lightning protector and a stand-alone solar-powered navigation light.

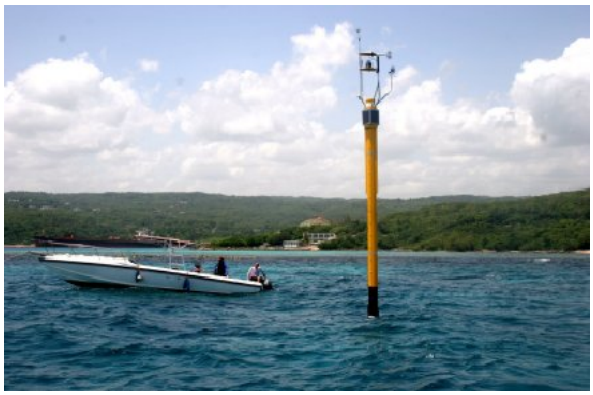

Figure 1. A CREWS Station in Discovery Bay, Jamaica.

Barometer (GE/Druck CS115)

The Barometer outputs a variable frequency which is measured by the data logger's period averaging instruction. It is mounted directly on the station's Control Unit (see below), inside the chamber at the top of the pylon

Wind Monitor, Electronic Compass (RM Young Models 05106, 32500)

The Wind Monitor measures horizontal wind speed and direction. It is connected to the data logger via the Electronic Compass, which accepts pulse counts and direction voltage as inputs and provides voltage outputs (updated once per second) for wind speed and corrected wind direction. Both instruments are mounted on an aluminum mast at 6.5 m above the ocean surface on the west side of the station. The Electronic Compass will be phased out during future maintenance visits to the stations.

Air Temperature Probe (Campbell Scientific Model 107)

The probe uses a thermistor to measure air temperature and is connected to one of the data logger's single-ended voltage channels, and is mounted near the top of the station out of direct sunlight, typically behind one of the station's solar panels.

Weather Transmitter (Vaisala WXT510)

The Vaisala Weather Transmitter (WXT) reports a wealth of meteorological measurements, some redundantly provided by other instruments on the station (air temperature, barometric pressure, wind speed and direction) and some not (humidity and precipitation amount, duration and intensity). Winds are measured acoustically by three ultrasonic transducers. Precipitation is also measured acoustically by a sensor that can distinguish between rain and hail. The WXT has a serial connection to the data logger by which it offers a full report once per minute, unprompted. It is mounted on an aluminum mast at 6.5 m above the ocean surface on the east side of the station.

Surface Light Sensor (Biospherical BIC2104R)

Biospherical Instruments' Multichannel Cosine Irradiance Profiling Spectroradiometers (BICs) are utilized for above water and underwater light measurements. The BICs used on CREWS stations are configured to take four kinds of light readings: three at discrete wavelengths of 305, 330 and 380 nm, and one across the Photosynthetically Active Radiation (PAR) range of wavelengths from 400 to 700 nm. The BICs additionally report low-resolution measurements of temperature and voltage. They communicate with the data logger via serial connections and are prompted for data once every 30 seconds. The surface BIC is mounted on an aluminum mast on the south-facing side of the station for the northern hemisphere.

Underwater Instrumentation

Underwater instruments include those which measure light, salinity, temperature and depth, as well as special deployments of instruments which measure the partial pressure of carbon dioxide and the fluorescent yield of corals. For added flexibility, all underwater instruments use the same type of connector (Impulse BH-4) and cable (multiply-layered with Kevlar, polyurethane, polypropylene and Vectran for maximum strength and resistance to fish bites and bio-fouling). The core instruments (Conductivity / Temperature / Depth sensors and underwater BICs) are doubled up with one set deployed "shallow" and one "deep," or at nominal 1 and 3 m depths. Such placement provides for redundant measurements, for calculating light attenuation, and to detect depth-dependent differences in temperature or salinity.

Conductivity/Temperature/Depth (Falmouth NXIC-CTD)

CTDs report measurements of conductivity, temperature, and pressure, and from these readings they calculate salinity, instrument depth and sound velocity. They also report instrument voltage and are equipped with flash memory and battery backup, and though they draw power from the CREWS station they continue to operate if the station is offline for maintenance. They power themselves up every 6 minutes, run for 30 seconds, and report averaged results from the run via RS-232 connections. They are mounted directly on the pylon at the same heights as the shallow and deep BICs. A simpler CT version of the same instrument is connected during station cleanings to provide ground-truth (i.e., validation) measurements.

Underwater BICs (Biospherical BIC2104U)

This is the same instrument as the Surface BIC (see above) except in a different housing. The underwater BICs are mounted on the sun-facing side of the station on extended arms to avoid light shading by other equipment.

SAMI pCO_2 (Sunburst Sensors)

Several Submersible Autonomous Moored Instruments (SAMIs) have been deployed on CREWS stations. One type of SAMI measures the partial pressure of carbon dioxide (pCO_2) and is important to

ocean acidification research. The SAMI pCO_2, being autonomous, is self-powered and logs its data to flash memory for later retrieval, but it also produces a report once per hour which is communicated to the station via RS-232 connection. This report includes a calculated pCO_2 value as well as sea temperature, blanking constants, and raw numbers for intensities at 434, 620 and 740 nm.

Monitoring PAM Fluorometer (Gademann Instruments)

The Pulse Amplitude Modulation (PAM) Fluorometer is an instrument that has seen several successful short-term deployments, most notably at the Bahamas CREWS station during the bleaching season of 2005 (Manzello et al. 2008). It consists of a central distributor box and multiple PAM heads that are deployed in near proximity to live coral specimens in the immediate area. The PAM, which has an RS-232 connection to the data logger, continuously measures a specimen's at-rest fluorescent response (F_o) and, once per hour, measures the response (F_m) to an intense burst of fluorescent light. The fluorescent yield calculated from F_o and F_m may be thought of as a measure of the specimen's photosynthetic efficiency, and the effects of environmental stressors may show up in these yield values before there are any visible signs of stress or bleaching.

Data Acquisition and Transmittal

Control Unit

The Control Unit is a collection of instruments that manage data acquisition and transmittal; this collection is supported by a fiberglass plate and Vectra for instrument and cable attachments. The Control Unit is thus a package that is installed in a hollow space at the top of the station pylon and is accessed by climbing to the top on removable rungs. Except for the navigation light, all station instruments connect to this unit, including surface and underwater instruments, antennae, batteries, solar panels, grounding wires and lightning protector. These connections are made via weather-proof plugs that are uniquely keyed according to purpose to eliminate the possibility of incorrect connections.

Data Logger (Campbell Scientific CR1000)

The data logger constitutes the "brains" of a CREWS station. It communicates with every instrument on the station via analog or serial RS-232 connections. It auto-calculates data averages, minima, maxima and totals. It is programmed to keep track of how many measurements it has received from each instrument and is capable of re-running complex instrument setup routines if communications fail. It summarizes its data once per hour for reporting via satellite (see Transmitter, below). All data are redundantly stored locally in its Compact Flash Module for later retrieval.

Transmitter (Campbell Scientific TX312)

The High Data Rate Geostationary Operational Environmental Satellites (GOES) transmitter is the main communications link between a CREWS station and the outside world. Each station has a 20-second window once per hour to send its data at 1200 baud. Data are currently transmitted plain-text but could be compressed to a binary format to make room in the future for more fields. The TX312 is connected to a satellite transmitter antenna and a GPS antenna on the outside, and has a direct connection to the data logger on the inside.

Radio (Campbell Scientific RF401)

Every station has a Spread Spectrum Radio for short-distance communications, used mainly for downloading data and for troubleshooting. The radio link is frequently used from a boat moored at the station, or from land-based locations within 500 m. Its antenna is mounted directly on the control unit.

Serial I/O (Campbell Scientific SDM-SIO4)

Instruments which communicate via serial RS-232 connections plug into a port on one of two 4-port interfaces which in turn connect to the data logger. Future control units may expand to include three such units (12 serial connections, total).

Power Supply and Peripherals

Batteries (Odyssey Drycell PC 1200 AGM)

CREWS stations use rechargeable dry cell Absorbed Glass Mat batteries. They are typically charged to between 12 and 14 V DC, with battery levels rising during the day when the solar panels are in sunlight and falling during the night. However, the station's power design allows it to continue operating without interruption for more than two weeks even without recharging its batteries.

Solar Panels (BP Solar SX 10M)

Each station has five 10 watt solar panels (i.e., 50 watts total) installed in a ring about the top of the pylon. This arrangement is partly to take advantage of sunlight from different directions throughout the day, but also to keep the panels as close to the pylon as possible to minimize the risk from the high winds of a tropical storm or hurricane. A Double Pole, Double Throw switch is installed between the batteries and solar panels to completely disconnect all power components prior to removing the Control Unit for maintenance.

Navigation Light (Carmanah Technologies Marine 601)

The station's navigation light is an important safety feature, as it not only provides warning of the station's presence but allows it to be used as a navigational aid that is tracked on marine maps and charts. It is completely stand-alone with its own battery supply and solar panels, and operates independently of station operations or downtimes. Each light is programmed with a flashing pattern that is identified on navigational charts.

Lightning Protection (Forestar Lightning Master Static Dissipater 151100; Wonder Bar Grounding Plate, Mark I)

All metallic and electronic parts of the station are connected by copper grounding wires which run down the inside of the pylon, then exit and terminate on a 15.2cm x 5.1cm x 12.7cm porous bronze grounding plate which is mounted underwater near the ocean floor. This keeps all equipment at the same electric potential and eliminates risk of shock to anyone touching more than one piece of equipment at the same time. Additionally, a lightning protector is integrated into this grounding system and is mounted at the highest point of the station above all other instrumentation. The lightning protector makes use of the point discharge principle to dissipate static charges before they can accumulate to the point where lightning streamers (a prelude to a lightning strike) can form.

Timing

Timing of events on the station is controlled by a main "scan block" of instructions repeated every five seconds. There is also a block of instructions executed exclusively at data logger startup. Table 1 depicts the series of events for data acquisition and transmittal.

Results

There are many challenges faced when deploying electronic equipment in ocean environments. Station operations may be impacted by marine life, equipment failure and tropical storm activity, among other challenges. Perhaps most crucial is the station's satellite communications system, since in the absence of regular data reports it is impossible to monitor instrument performance and environmental conditions.

The early years of the CREWS program (through 2003) saw several impacts from tropical storms and satellite transmitter failures. As a result, station "uptimes," defined as the percentage of time that stations are operational, held steady near 70%. With modern-era (2005 and since) CREWS stations, uptimes have held steady at 95% and above. In addition, in 2004 the design evolved to include an on-site memory storage module, so even in cases where transmissions have been lost due to weather or equipment problems, data are recoverable during the next maintenance visit. Taking into account this after-the-fact recovery of missing transmissions, station uptimes have fallen no lower than 98.9% since this modern instrument architecture was implemented in 2005.

Table 1: Timing of data acquisition and transmittal.

Timing	Events
startup	The logger program signature is recalculated. The logger clock is reset from GPS time. The transmitter is programmed with its GOES platform ID. Station instruments are initialized and/or programmed.
5 seconds	The logger program executes once. Meteorological instruments (air temperature, barometric pressure, wind speed/direction) are sampled.
30 seconds	Logger diagnostics are sampled. Light sensors are sampled (above and below the surface).
1 minute	The Vaisala Weather Station produces a full report. One-minute meteorological maxima, averages are calculated.
6 minutes	The CTDs produce their reports. The ground-truth CT, if connected, is sampled. The Monitoring PAM Fluorometer samples the at-rest fluorescent response (F_o). Transmitter diagnostics are sampled.
10 minutes	Ten-minute meteorological maxima, averages are calculated.
1 hour	The Monitoring PAM Fluorometer emits fluorescent flashes and measures the responses (F_m). The SAMI pCO_2 sensor produces a report. Hourly averages, maxima, minima are calculated. Error buffers, measurement counts are cleared. The station sends its hourly report via GOES (satellites).
1 day	The logger program signature is recalculated. The logger clock is reset from GPS time.

A graph of transmission uptimes is shown in Fig. 2. CREWS stations are identified by name in Table 2, which also shows "transmission uptimes" vs. "station uptimes." Transmission uptimes count the number of hourly satellite transmissions successfully received as a percentage of the total number of hours in the period,

whereas station uptimes describe the same dataset following the recovery of missing transmissions from the local memory module during a maintenance visit.

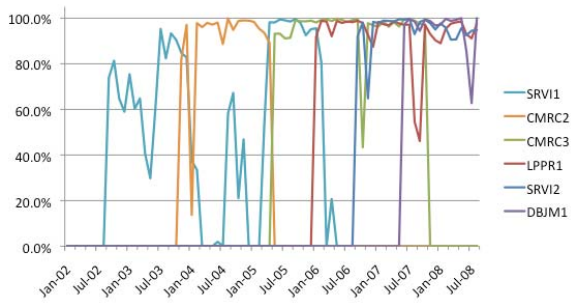

Figure 2. CREWS Station Uptimes, 2002 – present.

The graph shows the evolution of CREWS station performance. Earlier stations (CMRC2 and SRVI1) were of an intermediate pylon design and were prone to more frequent downtimes. The four stations installed since 2005 have much stronger uptimes and most of their outages can be traced to station upgrades or weather events. For example, the affect of Hurricane Dean on LPPR1 uptimes in August of 2007 is clearly seen. Note that one station (CMRC3) was placed on hiatus in October of 2007; its reinstallation is planned for late 2008.

Table 2: Transmission vs. Station Uptimes for CREWS stations, 2002 – present.

Station Name	Location	Station Lifetime	Transmit Uptime (%)	Station Uptime (%)
SRVI1	Salt River, St. Croix, USVI	2002/09 – 2006/06	55.5	80.7
CMRC2	Lee Stocking Island, Bahamas	2003/11 – 2005/04	91.4	93.8
CMRC3	Lee Stocking Island, Bahamas	2005/05 – 2007/10	95.7	99.4
LPPR1	La Parguera, Puerto Rico	2006/01 – present	93.0	99.4
SRVI2	Salt River, St. Croix, USVI	2006/09 – present	95.2	99.5
DBJM1	Discovery Bay, Jamaica	2007/06 – present	95.1	98.9

Discussion

As any marine scientist knows, the ocean is a harsh home for anything that does not live there, and this is why marine instrumentation is expensive and difficult to maintain over long periods of time. The ICON program has now been installing and maintaining stations that measure more than sea temperature for over eight years, and the experience gleaned over this time can serve to save valuable time and resources for other agencies and organizations wanting to conduct similar endeavors. The high quality data we have been able to collect has been used for important research findings (e.g., Hendee et al. 2002; Manzello et al. 2006, 2008) which would have been difficult to obtain without the architecture reported here.

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CoralWatch – a flexible coral bleaching monitoring tool for you and your group

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Abstract. The CoralWatch Coral Health Chart, a standardised colour reference card, is an inexpensive, flexible tool that anyone can use for rapid, wide-area assessment of changing coral condition. The charts are used in a growing number of scientific studies as well as by a large number of non-expert visitors to reefs world-wide. Here we describe and analyse two general ways in which the methodology can be used to provide useful data on coral reef condition over time, 'fingerprinting' and long term monitoring of selected colonies. Data stemming from trained and untrained observers, collected via the CoralWatch online database is analysed and compared to data collected by trained observers. Detection of bleaching events and evaluation of recovery rates of corals against temporal and geographic variation in coral colour requires more data collected regularly and frequently by anyone using this globally applicable method.

Key words: colour, coral bleaching, recovery, reef fingerprint, volunteer

Introduction

The CoralWatch Coral Health Chart, a standardised colour reference card, is an inexpensive, flexible tool that anyone can use for rapid, wide-area assessment of changing coral condition (Siebeck et al. 2006). Long-term trends in bleaching and recovery are not well understood at present due to the limited data available. What has been lacking is an objective method that can be used over large spatial scales by volunteers with limited or no training. Various other observer-based methods exist, however, they often require rigorous training and are thus restricted to a relatively small number of scientific volunteers (Miller and Müller 1999).

Measurements with the Coral Health Chart rely on observers finding the closest match between a coral and a colour on the chart. Even with limited training observers are able to do this accurately (Siebeck et al. 2006). Any visitor to a reef can contribute valuable information by simply following the instructions on the card and adding their data to the CoralWatch online database. On the other hand, the chart has been accepted as a useful tool for scientists and is regularly used to assess coral colour or condition (Fabricius 2006; Anthony and Kerswell 2007; Cox 2007; Frisch et al. 2007; McClanahan et al. 2007; Cooper et al. 2008; Spalding 2008).

Here, two general ways of using the charts are discussed, 'reef fingerprinting' and long-term monitoring of individual colonies. We also present a preliminary analysis of the dataset collected through the CoralWatch website.

Material and Methods

Colour measurements

Coral colour was determined as described in Siebeck et al. (2006). Briefly, the Coral Health Chart was held near the selected coral colony. The chart was rotated until the closest match between the overall colour of the colony and a colour on the chart was found. In all cases, the colour score (1-6) of the matching colour was used for the analysis. In previous work a controlled bleaching experiment was used to calibrate the chart with respect to symbiont density and chlorophyll a (Siebeck et al. 2006). It was found that a colour score difference larger than 2 indicates a significant change in symbiont density and chlorophyll a content, and thus bleaching state.

Long-term monitoring of tagged colonies

Twenty randomly selected colonies (10 bleached colonies with colour score < 2, and 10 non-bleached colonies with colour score of > 3 of various species) were marked on the Heron Island Reef flat, Great Barrier Reef, Australia with plastic tags during the 2002 bleaching event. Colour chart measurements were made once every two weeks for 24 months. In each case the observer (Heron Island Research Station Staff) recorded the lightest area excluding the tips.

Fingerprinting

Two independent trained observers collected colour data for 100 randomly selected corals on the Heron Island Reef. Their instructions were to randomly

select a coral in the inner reef flat, measure its colour, then take three steps towards the reef rim, identify the coral closest to them and record the colour of that coral. The observers were instructed to repeat this selection process until 100 corals were measured. For each coral, the observers noted both the lightest and darkest area excluding the tips of branching corals. Data were collected in this way during the 2002 bleaching event (Heron Island, March) and during the winter after the bleaching event (Lizard Island, Green Island and Heron Island, July).

Volunteers using the Coral Health Chart followed the instructions on the chart (as above) and entered their data via the CoralWatch website. Any suspected mistakes in the dataset were removed before analysis. The following information in the dataset was used to detect mistakes: entries labeled with the words "test" or "practise", exact replicates of nearby entries, entries of corals with exactly the same colour score, or entries missing critical data fields, such as 'location', 'date' and 'colour scores'. The data of all volunteers that entered at least 10 datapoints were included. The cut-off was set arbitrarily to ensure that the volunteer had reasonable experience with the method and shown some enthusiasm for it.

Long-term datasets for sites in Australia (Heron Island) and Netherlands Antilles (Barracuda Reef and Mushroom Gardens, St. Eustatius) were examined for changes in coral condition over time. A comparison was also made between coral colour data from a site with reported bleached corals (Christmas Island, April 2005) and data from the same site monitored on different dates. Colour scores from different sites (Heron Island, North Keppel Island, Australia and Richelieu Rock, Thailand March 2005) monitored around the same time as the bleaching occurrence were also examined.

Results

Long-term monitoring of 20 coral colonies

Repeated colour measurements of the 20 marked colonies on the Heron Island Reef flat document the recovery process the corals went through following the 2002 bleaching (Fig. 1a). Pair-wise comparisons show that until May there is a significant difference between the group of corals that appeared healthy (group 1) and the group that was visibly affected by the bleaching (group2) (March & early April $F_{1,18}$ >56 p<0.0001; late April $F_{1,18}$ > 16.0, p = 0.001; 8-May: $F_{1,18}$ = 3.1, p = 0.097). As the corals recover this difference disappears and the colours of both groups are similar – although there is a trend for bleached corals to maintain a lower colour score.

In early September, a second sampling period was initiated as the observers noticed minor bleaching after a severe rainstorm. Results show that both groups of corals suddenly bleached again and further recordings document their recovery from that bleaching event. During the event the colours of both groups are similar. The colour before and after the rainstorm is significantly different for group 1 (ANOVA, $F_{1,6}$ = 21.1, p = 0.004) but not for group 2 (ANOVA, $F_{1,12}$ = 3.3, p = 0.097). During the winter months slight fluctuations of the colours of both groups can be observed.

Examples for the variability in recovery rates and timing are shown in Fig. 1b. Colonies 1-3 start to recover at different times within the first month of sampling, while colony 4 does not recover within this first observation phase. At the beginning of the second observation phase colony 4 and 1 show some recovery (colour change of 2-3 scores) until November followed by colony 3 in early December. Interestingly, colony 2 which showed fast recovery in observation phase 1 does not seem to recover from the rain bleaching. This is an example of how the fate of individual corals can be mapped.

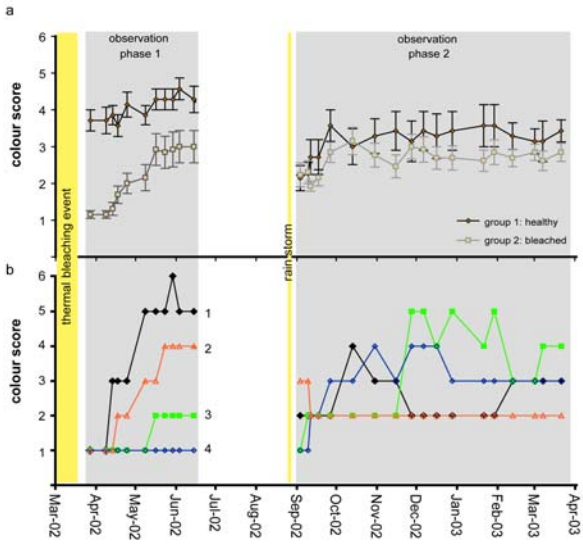

Figure 1: Results for the repeated colour measurements of the 20 corals on the Heron Island Reef Flat following the bleaching event in early 2002 and a second bleaching event caused by a rainstorm at a low tide. a) The average colour scores (mean ± se) are given for healthy corals (group1) and bleached corals (group2). b) Colour change of 4 example colonies showing variability of responses during the observation phases following the two bleaching events.

'Fingerprinting' the reef

Trained observers

The distribution of coral colour on the Heron Island Reef flat during the 2002 bleaching is significantly different from the distribution measured in July (ANOVA, $F_{1,237}$ = 339, p < 0.0001; Fig. 2a). In March, the distribution is skewed and most corals have a

colour code of 1, which represents white coloration. In July, the frequency distribution appears more normal and very few corals have a colour code of less than 3.

A comparison of the colour of 438 corals around the Heron, Green and Lizard Island Reefs in July 2002 shows that the colours on the three reefs have a similar distribution (Fig. 2b). There is no significant difference between the colour scores of corals from Lizard Island and Green Island during July 2002 (ANOVA, $F_{1,259} = 0.098$, $p = 0.8$) while the colours of the Heron Island Reef in the same month are significantly darker than those of the other two reefs (ANOVA, $F_{2,435} = 5.4$, $p = 0.005$).

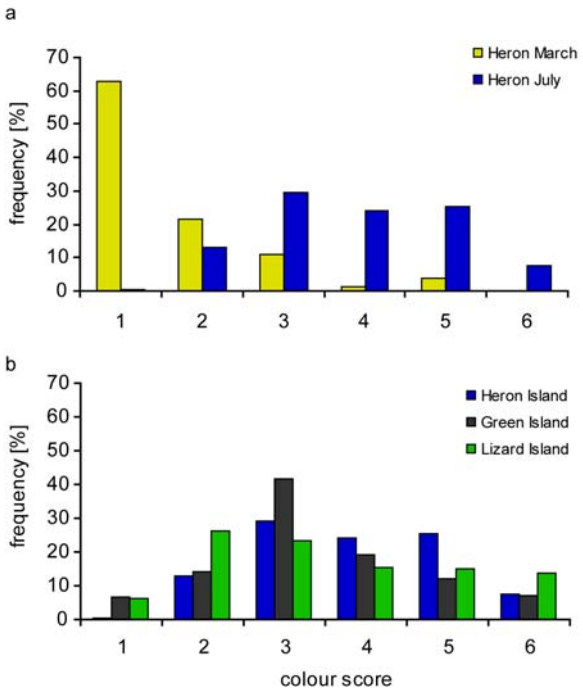

Figure 2: Results of the 'fingerprinting' survey. Distribution of coral colour a) on the Heron Island Reef flat during (March) and after (July) the 2002 bleaching event and b) for a total of 438 corals on the Heron, Green and Lizard Island Reefs in July 2002.

Untrained observers (online database)

Data from 246 sites in 44 countries have been collected and reported by 440 different volunteers (CoralWatch 2008). A bleaching event was reported anecdotally in April 2005 at Christmas Island. Average colour scores were found to be measurably lower (~2 scores) than those observed in October 2005 and October 2006 at the same site (Fig. 3a). Scores were also lower than those from Heron and North Keppel Islands (Australia) and Richelieu Rock (Thailand) measured one month before (Fig. 3b).

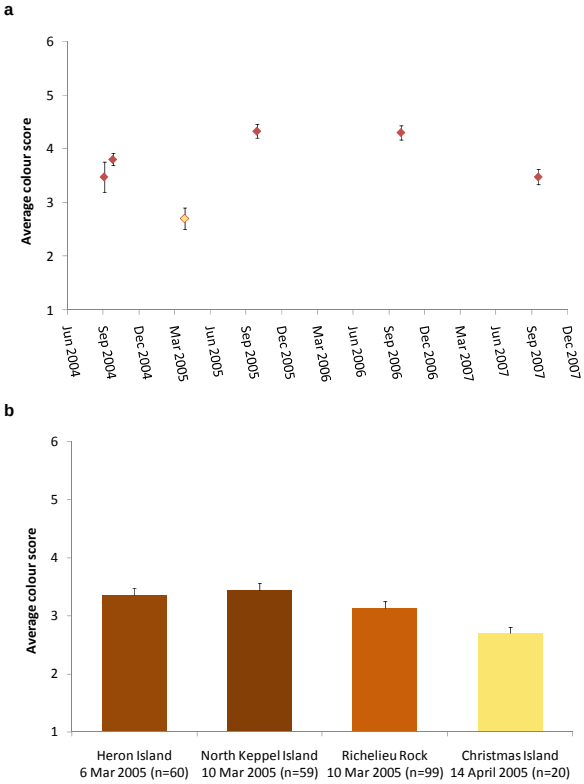

Figure 3: Results of volunteer 'fingerprinting' surveys. Average daily coral colour scores for a) all days that monitoring occurred at Christmas Island sites and b) Heron Island, North Keppel Island (Australia) and Richelieu Rock (Thailand) in March 2005 and Christmas Island in April 2005. Note: Brightness of colours shown using a brown hue in these two graphs provide a rough approximation of the actual average coral colour scores measured.

The largest datasets stem from 3 reef sites, Heron Island (Australia) as well as Barracuda Reef and Mushroom Gardens at St Eustacias, (Netherlands Antilles). Corals at Heron Island Reef observed between 2003 and 2008 show a slight trend of lighter coral colours in spring (Sep to Nov) and darker colours in autumn (March to May) (Fig. 4a). Irregular monitoring occurred in some years, with frequency of data collection varying throughout the year. Data that has been collected more frequently and regularly at both Heron Island (May to Oct 2003) and the Netherlands Antilles show less fluctuation in coral colour over time (Fig. 4b and 4c).

Some slight variation between sites is seen in regard to overall average colour scores, with the coral colour at Heron Island Reef being darker than both Barracuda Reef and Mushroom Gardens (Fig. 4d).

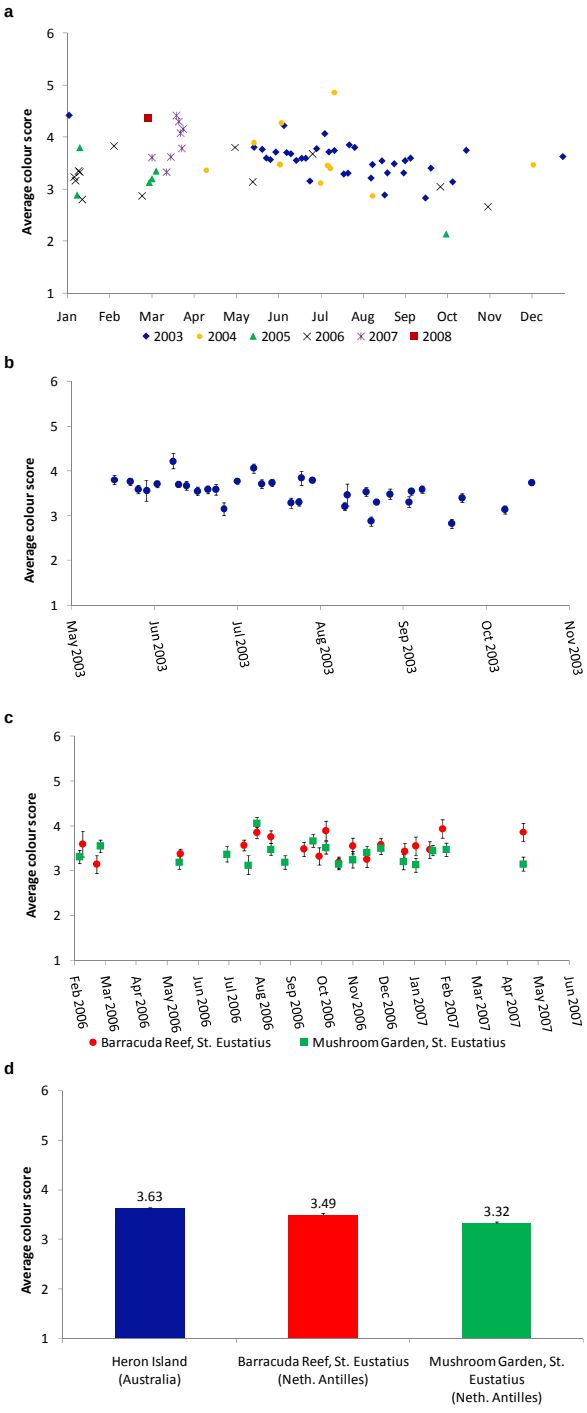

Figure 4: Long term data sets of volunteer 'fingerprinting' surveys. Average daily colour scores (mean ± se) are shown for a) all days that monitoring occurred at Heron Island, displayed over an entire year from 2003 to 2008, b) Heron Island from May to October 2003 and c) Barracuda Reef and Mushroom Gardens (St. Eustatius, Netherlands Antilles) from February 2006 to April 2007. d) Overall average colour scores for long term data at Heron Island, and St. Eustatius.

Discussion

The CoralWatch Coral Health Chart provides a six point scale with which changes in coral colouration, as an indicator of symbiont density, can be measured. This scale can be incorporated in any existing monitoring methodology. Here, an example of two methods proposed to be useful for the wider community are presented: 'fingerprinting' of randomly selected corals and long-term monitoring of specific colonies. The former method leads to large-scale information about the general condition of a reef while the latter method can be used to detect condition changes of individual colonies. At present, the fingerprinting method is better suited to untrained volunteers, such as tourists as even a single visit to the reef can yield useful data when added to the general database. In the future, marked coral colonies, along permanent transects, will provide the opportunity for anyone to participate in long-term monitoring of specific coral colonies. Benefits gained by involving non-scientists are not only due to large amounts of data gathered but also include education and an increase in general awareness of environmental problems, such as global warming and its consequences (Pfeffer and Wagenet 2007).

Repeated measurements of 20 colonies on the Heron Island Reef flat allow the documentation of the condition change of a group of corals, some of which were affected by the 2002 bleaching event. Within six weeks of the first measurement, most bleached corals had recovered and their colours were similar to those of the healthy corals. Two corals had not recovered at that time, which is why the average colour score of the bleached groups is lower than that of the healthy group. It is interesting to note that even the corals that appeared healthy during the bleaching event show a slight darkening in the months following the bleaching, and it is possible that they were also affected by the bleaching event. On the other hand, the possibility exists that the darkening is due to annual changes in symbiont density (Fitt et al. 2000).

The data for individual corals indicate that different recovery timeframes exist. Indeed, there is evidence that coral species have different temperature and time thresholds at which they bleach (Berkelmans and Oliver 1999; Marshall and Baird 2000; Berkelmans 2002) and that this may be true for recovery as well (Hueerkamp et al. 2001). However, more data are needed to detect species-specific patterns.

The first survey period ended in winter, when it appeared that the coloration of the corals would remain relatively constant. However, the same observers noticed that the reef appeared bleached after a severe rainstorm and initiated the second survey period. Both original coral groups were affected, and it appeared that the corals belonging to the group classified healthy during the March

bleaching was actually affected more than the previously bleached group. On average, recovery of both groups was faster after the rain bleaching compared to the bleaching in March, presumably because the salinity returned to normal within just a few hours. This is a good example of how non-expert observers can use the charts to quantify changes in coral health.

Unlike the long-term monitoring of specific coral colonies the 'fingerprinting' protocol is not dependent on the presence of permanent transects and is thus suitable for any interested visitor on any reef worldwide. The 'fingerprinting' method is able to pick up differences between as well as within bleached and recovered reefs. It appears that corals on southern reefs (Heron Island) are relatively darker than corals on northern reefs (Green Island and Lizard Island), and that corals on the Heron Reef flat had made a significant step towards recovery by July.

Untrained volunteer observers collecting CoralWatch data provide a measure of coral condition over time, increasing the chance that a bleaching event will be detected while supplying baseline data that highlights temporal or geographic variation in coral colour. Bleaching events, such as those at Heron Island and Christmas Island, are evident as lower average colour scores when compared against this baseline data.

These data are publicly available and can be reported and used by anyone to make general comparisons of coral condition. Correlations can be made with recorded time of day, weather conditions, coral types, sampling activity and water temperature (CoralWatch 2008), as well as with information from other complimentary datasets on reef condition, such as Reefbase, Coral Reef Watch or Reef Check.

A measure of long-term fluctuation in coral condition is provided in better resolution by following individual colonies over time and could include volunteers observing permanent transects at select sites. Schools and tourism operators returning regularly to the same sites are well suited to monitoring long term trends in coral colour change. Hundreds of recreational divers have registered through Project AWARE and are including CoralWatch monitoring in their dive training and regular dive trips. Other uses of the CoralWatch method include impact assessments, as are being performed by OceanWay in Hong Kong to measure the potential effects of dredging on corals.

More data collected on a regular basis are needed to better detect trends and identify variables influencing changes in coral colour relating to coral bleaching and recovery and potential natural cycles (for example, see Fabricius 2006). Involving more people in consistent observations or targeted reef monitoring in response to predicted or detected bleaching events is expected to improve the current sporadic or non-existent nature of volunteer and scientific monitoring at many reefs sites.

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Testing the generality of acoustic cue use at settlement in larval coral reef fish

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Abstract. Some settlement-stage larval fish appear to be attracted to reef sound and may, therefore, use acoustic cues when orientating towards their settlement site. However, all work on the *in situ* response of coral reef fish larvae to sound in acoustic playback experiments has been carried out in the same location (Lizard Island, the Great Barrier Reef), and in some cases, using the same reef recording. It is therefore not clear how widespread acoustic cue use is. To test whether sound is a general and reliable indicator of reef settlement site, we conducted a similar experiment in a different coral reef region, where the coral reef habitat and therefore soundscape is less uniform in quality (Bohol, Philippines). Contrary to our predictions, in some cases we found that fish were not attracted to the broadcast reef sound. We suggest that this may be due to an artefact of the reef recording, possibly the location or the time of day the recording was made. Our results indicate that larval fish are more selective in their response to coral reef sound rather than just being innately attracted to generic reef sound. This highlights the need to assess anthropogenic impacts on the natural soundscape, as this could affect the ability of larval coral reef fish to acoustically detect a suitable settlement site.

Key words: coral reef fish, cue, sound, light traps, settlement

Introduction

Ten years ago, it was hypothesised that larval reef fish could use sound to locate a settlement site (Stobutski and Bellwood 1998). There are now data that show that as early as the embryonic stage, coral reef fish can detect sound and their sensitivity to sound increases with age (Egner and Mann 2005; Kenyon 1996; Simpson et al. 2005a). At the time of settlement, damselfish (*Pomacentrus nagasakiensis*) are as equally sensitive to sound frequencies as juvenile-stage fish, and therefore are physiologically able to receive acoustic information (Wright et al. 2005). Additionally, Pomacentridae larvae can determine the direction of a sound source and will swim towards reef recordings broadcast in a choice chamber (Leis and Lockett 2005; Tolimieri et al. 2004). This is not just a general phonotactic response but appears to be specific to reef sound as fish were attracted towards reef recordings, but not artificial pure tones (Leis et al. 2002).

Acoustic playback experiments have shown that reef fish are attracted to light traps broadcasting reef sound over the ambient soundscape (Leis and Carson-Ewart 2003; Simpson et al. 2004; Tolimieri et al. 2000), and higher natural settlement rates are seen on patch reefs that were associated with underwater speakers playing reef recordings, in comparison to silent control patches (Simpson et al. 2005b). Generally, settlement-stage fish are more attracted to the higher frequency components of reef sound (made predominantly by invertebrates), relative to the original recording and the filtered lower frequencies alone, so sound appears to be more than just a broad indicator of reef location and may provide specific information used in settlement site selection (Simpson et al. 2008).

The use of sound for orientation during settlement varies among families, however, with some families appearing not to respond to sound cues (Leis and Carson-Ewart 2003; Simpson et al. 2004). What is not yet understood is how widespread acoustic cue use is. With the exception of one study carried out on sub-tropical rocky reef fish (Tolimieri et al. 2000), the remaining seven *in situ* studies that have shown positive phonotactic responses of larval fish to coral reef sound were all carried out at Lizard Island. Four of these studies shared the same single reef recording as the test sound (Simpson et al. 2004; Simpson et al. 2005b; Simpson et al. 2008; Tolimieri et al. 2004), and the remaining three used another (Leis et al. 2002; Leis and Carson-Ewart 2003; Leis and Lockett, 2005). As a result, our knowledge of acoustic cue use

in settlement-stage fish orientation is potentially very location specific and it has not been investigated in any other coral reef area, where the soundscape may be less consistent due to variability in reef quality. We questioned the generality of acoustic cue use by testing the response of larval coral reef fish to sound in a different location. Using the same techniques that have previously been used to assess the attraction of settlement-stage fish to broadcast reef sound at Lizard Island (i.e. coupling light traps with underwater speakers), we carried out a similar experiment on settlement-stage coral reef fish in the Philippines. Light traps collect phototactic larval reef fish at the end of their pelagic phase, and the comparison of catch rates in the sound treated vs. the silent traps can be used to assess the attraction of settlement-stage fish to the broadcast sound treatment (Leis et al. 2003; Simpson et al. 2004; Simpson et al. 2008; Tolimieri et al. 2000). We predicted that if sound is a general and reliable indicator of reef location, it will be used by settlement-stage fish in this different study area, therefore higher numbers of fish would be attracted to the sound, in comparison to the silent control treatment.

Methods

Traps (designed by Ecocean, St Clément de Rivère, France) were set at surface moorings located in a sea channel to the northeast of Pangapasan Island, Bohol, central Philippines (10°01.1'N, 123°56.2'E). The moorings were anchored on a sandy substrate in water of 10-12 m depth. There was no reef present within 50 m of each mooring and they were separated by c. 400 m to prevent acoustic overlap of the different traps broadcasting sound. The area at which the broadcast sound was detectable over the ambient reef sound was estimated to be 20-50 m (see Fig. 1).

Each night, two sound and two silent traps were each pseudo-randomly assigned to a mooring, so each treatment was tested multiple times at each position during the experiment. The sound systems consisted of an MP3 player, a 12V lead-acid battery, 18W Universal Amplifier Module (Kemo-Electronic GmbH, Lanhen, Germany), and an Electrovoice UW30 underwater speaker (Lubell Labs, Columbus, OH, USA). This played the sound treatment on continuous loop through the night, which was a recording taken at 8.40am on the 16th June 2007, at Black Forest Reef, a marine protected area located to the southwest of Bohol (09°31.228'N, 123°40.991'E). The recording was taken using an Edirol R1 recorder, and a HTI-96-MIN omni-directional hydrophone with a built in preamplifier (High Tech, Inc., Gulfport, MS, USA) and processed using Audacity 1.2.6. (a free digital audio editor available at http://audacity.sourceforge.net/) to delete artificial artefacts (e.g. the sound of distant boat engines) and produce a clean one-minute recording.

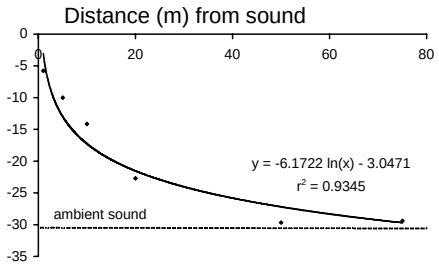

Figure 1. The distance over which the sound treatments were detectable over the ambient sound level. Sound intensity (root mean squared measured in relative dB) was measured at increasing distances from the sound system playing back a pure tone sound.

The experiment was conducted over 21 nights from the 4th-24th July. Traps were deployed at dusk, left overnight and collected at dawn, when the catches were transferred to separate polystyrene cool boxes and transported by boat to a nearby aquarium facility in Matabao, Bohol. Reef fish were separated from the rest of the catch (which consisted primarily of invertebrates and clupeids) and identified to family, or when possible, species level and counted. The fish were then given to Ecocean for rearing for a release scheme.

Analysis

There are no data available on the behaviour of larval fish upon entering light traps, therefore we do not know if it is a fair assumption to treat each captured fish as a statistically independent data point. For this reason, two approaches were taken for the analysis. A sign test, which makes no assumptions on the independence of fish caught, was used to test the null hypothesis that the number of nights with the largest catch would be the same for the silent and sound treated traps. As this test has a low power to detect a treatment difference when the number of testable nights per family is low (after excluding ties), the second approach estimated the effect of the sound treatment on the number of larval fish caught by fitting a generalised linear mixed effects model (GLMM). This method does assume larvae entered the trap independently, however it has the benefit of including the temporal and spatial variation that is characteristic of larval fish distribution and occurrence in light traps. Counts were grouped by family and families for which fewer than 10 individuals were captured over the experiment were excluded. Counts of fish per family were not normally distributed (Shapiro-Wilk test, $W = 0.1878$, $p < 0.001$). A logarithmic link function and Poisson

error distribution was specified as the data set was bounded by zero and the variance in counts per family was not equal. As there was inter-family variation in abundance, the number of fish caught per mooring and the number of fish caught per day over the lunar cycle, these were fitted as random effects. An interaction between sound treatment and family was fitted as a main fixed effect. As a significant trap unit effect was not found it was dropped from the model. The models were fitted using maximum likelihood.

Deviance statistics (estimates of how well the model captures the data) were generated for each model with and without the explanatory variables. To obtain the significance levels of the explanatory variables, the deviance statistics were compared using Chi-square tests. All analyses were implemented in R (R Development Core Team 2007).

Results

Twenty nights of data were collected, with 78 trap deployments (39 sound and 39 silent) as bad weather (on the 8th night) caused all traps to be retrieved early. A sound system failed on one occasion and on another a mooring was stolen, preventing a silent trap from being set, leading to a total of 18 nights data with sound treated and silent control trap deployments.

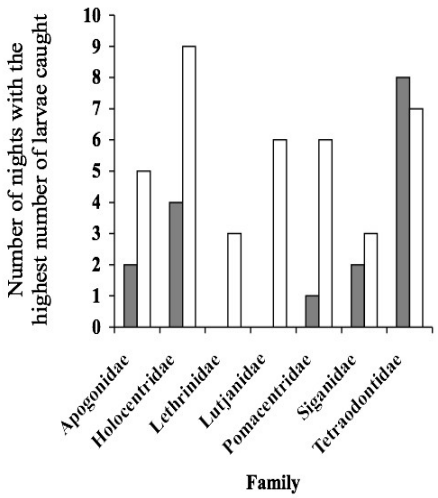

Figure 2. Number of nights with the greatest catch per treatment deployed with speakers (grey) and without (white) from the 4th-24th July 2007, Bohol, the Philippines.

A total of 326 larval coral reef fish from 14 families were caught (see Table 1). The four most common families (Apogonidae, Holocentridae, Siganidae and Tetraodontidae) comprised 75% of the total catch.

Table 1. Summary of catches of settlement-stage coral reef fish larvae caught in light traps with broadcast reef noise (sound) and without (silent). Low catch rates prevented analysis of some of the families. Results (significance levels) of the sign tests (per family) and the generalised linear mixed effects model (GLMM) (families grouped according to their direction of response to the sound treatment) are shown (see methods for details).

Family	Silent	Sound	Total	Sign test	GLMM
Apogonidae	31	12	43	0.226	
Holocentridae	22	13	35	0.133	
Lethrinidae	8	2	10	0.125	< 0.001
Lutjanidae	14	4	18	0.015	
Pomacentridae	13	1	14	0.062	
Siganidae	57	26	83	0.500	
Tetraodontidae	45	44	99	0.500	0.718
Blennidae	1	1	2		
Carangidae	2	2	4		
Chaetodontidae	0	1	1		
Mullidae	2	5	7		
Scaridae	0	2	1		
Sphyraenidae	1	4	5		
Syngnathidae	2	2	4		

Sign tests showed that one family (Lutjanidae) was caught in greater numbers on significantly more nights in the silent traps (see Table 1; Fig. 2).

In contrast, the less conservative GLMM that takes into account other spatially and temporally variable factors found that there was a variable response of fish families to the sound treatment (family: sound treatment interaction, χ^2: 14.41, p= 0.025). When the seven most abundant families were grouped according to their direction of response to the sound treatment, six (Apogonidae, Holocentridae, Lethrinidae, Lutjanidae, Pomacentridae and Siganidae) were caught in higher numbers in the silent traps in comparison to the sound (χ^2: 15.240, p<0.001). There was no difference between the sound and silent treatment in catch rates for the Tetraodontidae (*post hoc* Mann-Whitney, W: 118.5, p= 0.718).

Discussion

Settlement-stage larval fish were not attracted to the broadcast reef sound. We predicted that if fish could detect and were attracted to reef noise, there would be higher catch rates in the sound treated light traps. The opposite effect was found for the Lutjanidae, where significantly more fish were caught in the silent than in the sound treated traps. There was an overall

trend, when the abundant families were grouped together, for higher catch rates in the silent treated traps. This result is in contrast to those from four previous acoustic playback studies in which fish (of the families caught in this study: Apogonidae, Holocentridae, Lethrinidae and Pomacentridae) were attracted to broadcast reef sound (Leis and Carson-Ewart 2003; Simpson et al. 2004; Simpson et al. 2005b; Simpson et al. 2008). Our results are consistent with those of Leis et al. (2003), who demonstrated that the attraction of settlement-stage apogonids and pomacentrids to sound varied with location. In that study larvae responded positively to the sound treament at inshore but not offshore sites. There are two possible explanations for the lack of congruence with the findings that settlement-stage fish are attracted to reef sound: 1) there was a negative effect between the design of the traps used and the sound treatment 2) there was an artefact of the recording we broadcast for the sound treatment that acted as a repellant to settlement-stage larval fish.

We used a light trap that has a more open entrance than did those used at Lizard Island. This could mean that fish entering the trap were more vulnerable to predation. So if for example, the sound treatment also attracted predators, this could reduce the number of fish caught. Without any data on the rate of predation on fish entering the trap, this explanation, as with any other on a potential trap type and sound treatment interaction, is speculative. However, this is unlikely to have contributed to our finding that settlement-stage fish were caught in higher numbers in the silent traps, as when the Ecocean traps were used at Lizard Island in 2007-8, the most commonly caught families were more abundant in the sound treated traps (Heenan, pers. obs). This asymmetry also is unlikely to be the result of the fish caught in our study being unable to detect the sound treatment, as if this were the case, one would expect an equal number to be caught in the silent and sound treated traps. Without further experimentation, we do not know if this represents a general avoidance of coral reef fish larvae to sound in this region of the Phillipines or if it was specific to the recording used for the sound treatment.

There are two aspects of the recording itself that may have been repellent to settlement-stage coral reef fish. The first concerns the variation in reef sounds: they vary with time (season, moon phase and time of day); and the biological chorus has cyclical patterns in intensity, peaking during summer evenings around the new moon (Cato 1978; Radford et al. 2008). This coincides with when larval fish arrive in highest density to recruit to the reef (Dufour and Galzin 1993; Irisson and Lecchini 2008). While settlement-stage fish are attracted at night to nocturnal reef recordings (Leis and Carson-Ewart 2003; Leis and Lockett 2005; Simpson et al. 2004; Simpson et al. 2005b; Simpson et al. 2008; Tolimieri et al. 2004), they do not respond to nocturnal reef noise during the day (Leis et al. 2002; Tolimieri et al. 2004). Due to logistical reasons, the test recording we used was taken in the morning (8am), however *in situ* observations of released larvae showed that they orientate away from the reef during the day in Australia (Leis and Carson-Ewart 2002). In this study larval fish were repelled by a daytime recording, therefore this result supports the diel dependent nature of larval attraction to sound, suggesting that 1) they can perceive the difference between the sound of a reef at night and during the day and 2) they use this information to time their approach to the reef.

The second aspect of the test recording relates to the difference between the area where it was taken and played back. Located 60 km away, the recording was chosen as it was a marine protected area, with high fish diversity and abundance, and we believed it to be a biologically rich in sound. Some settlement-stage larval fish appear to imprint to their natal reef site by olfaction (Arvedlund and Nielsen 1996; Arvedlund et al. 1999; Gerlach et al. 2007), and so as embryonic stage fish can hear sounds (Simpson et al. 2005a) it is plausible to suggest that imprinting may also occur to natal reef sounds. If this were the case in this study fish may have been affected by the non local aspect of the test sound. However, in four separate studies, larval fish at Lizard Island were attracted to a recording taken at Feather Reef, which is located over 300 km away, which shows that larval fish will respond to a non local recording. Instead, we suggest that the test recording sounded sufficiently different from the ambient acoustic conditions that were characteristic of the playback site (a channel flanked by two reefs that had degraded to urchin and algal dominated reefs), that it caused fish in this area to avoid the played back sound.

This is the first *in situ* acoustic playback experiment on settlement-stage coral reef fish performed outside of the Great Barrier Reef, and we found that in contrast to these previous studies, catches of larvae decreased due to the sound treatment. This suggests that larvae are more selective in their response to reef sound, rather than having a generic innate attraction. Given the potential for habitat degradation, overfishing and anthropogenic sources of sound to modify the natural soundscape, acoustic surveys are needed to compare the soundprints of different reefs. Furthermore, experiments are required to determine the selectivity of acoustic cue use in settlement-stage fish, as it seems possible that this could affect the

ability of larvae to acoustically detect a suitable settlement site.

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A glimpse of the Florida Area Coastal Environment (FACE) program

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Abstract. The Florida Area Coastal Environment (FACE) research program gathers a variety of data related to water inputs into the coastal zone of southeast Florida. The water inputs studied include treated wastewater discharges, inlet flows, and upwelling events. Measurements include currents, nutrients, microbial contaminants, and stable isotopes. This report provides a glimpse of the data collected in this program. Data collected from the Boynton inlet point to the significance of this discharge as a source of nutrient and microbiological loads to coastal waters and demonstrate the importance of accounting for all major discharges in order to fully understand the impact of land use and water management decisions on coastal resources.

Key words: wastewater, inlets, Florida, nutrients, microbial contaminants.

Introduction

The Florida Area Coastal Environment (FACE) program is an ongoing research effort lead by the NOAA's Atlantic Oceanographic and Meteorological Laboratory in Miami, FL since 2004. The broad objectives of the studies include: (1) quantify the sources of selected nutrients and microbial contaminants, (2) ascertain the relative contributions of those sources to the nutrient budget and microbiologic loads of the region, and (3) determine the likely exposure of certain coral reef resources to those nutrient and microbiologic sources. The study area of FACE covers 364 km of coastline in the counties of Miami-Dade, Broward, Palm Beach, and Brevard, and includes six treated wastewater plants (TWWPs): Miami Central, Miami North, Hollywood, Broward, Boca Raton, and South Central (Fig. 1), which together contribute ~1 million cubic meters (284 millions of gallons) per day to the region. In addition, this coastal area receives fresh water discharged through six inlets, from the Miami Harbor inlet in the south to the Boynton inlet in the north (Fig. 1).

The FACE field campaigns have included inlet and outfall plume tracer studies, nutrient surveys, ocean current measurements via ADCP, microbiological survey studies, and stable isotope studies on a variety of research vessels (NOAA's R/V Cable and R/V Nancy Foster, R/V Coral Reef II, and the University of Miami's R/V Walton Smith). These field programs are outlined in Table I.

Material and Methods

Tracer Studies. An outfall tracer study was conducted on the Hollywood outfall in June 2004 by injecting sulfur hexafluoride (SF_6) gas into the outfall pipe over the course of six days. Methods for this study are available in Wanninkhof et al. (2005). A second outfall tracer study was performed near the outfall of the South Central Regional Waste Water Treatment Plant (SC) in 2007. In the latter study, rhodamine-WT dye (RD) was introduced into the effluent flow for a 48-hour period commencing on 26-Feb-2007. RD was also introduced into an outgoing tidal flow in the Boynton Inlet (26°32'43" N, 80°2'30"W), on 22-Feb-2007 to track the discharge of the inlet. The RD was tracked by ship using a towbody equipped with a hose and a pumping system to obtain water samples. Two sensors (YSI model 6600, for temperature, RD, chlorophyll, salinity, and

depth) were moored at the bottom and at mid-water above a location on Gulfstream Reef.

Inlet Intensive Studies. In addition to the tracer study at the Boynton Inlet, two 48-hour intensive water sampling periods were conducted at that location. The objective was to collect water samples over four complete tidal cycles in order to investigate the nutrient and microbial loads contributed by this inlet. The first sampling event was conducted June 4-6, 2007 and the second on September 26-28, 2007.

Table 1. FACE field programs.

Study Name	Goals	Study Area	Platforms	Notes	Dates
Hollywood Tracer	Define HW outfall plume 10-66 km	HW outfall	R/V Coral Reef II	Rhod-amine dye, SF_6	7-9 June 2004
Outfalls Survey 1	Survey of all six TWWP outfall plumes	all outfalls	R/V N. Foster	Nutrients, multibeam, microbio	6-19 Oct 2006
Outfalls Survey 2	Survey of all six TWWP outfall plumes	all outfalls	R/V N. Foster	Nuts, bio, isotopes	8-16 Feb 2008
FOCITE-1	SC outfall / Boynton Inlet tracer experiment	SC plume, Boynton Inlet	R/V Coral Reef II	Winter	19-22 Feb 2007
FOCITE-2	SC outfall / Boynton Inlet tracer experiment	SC plume	R/V Walton Smith	Summer	10-13 June 2008
Boynton Intensive-1	Water, Chem & Bio Flux thru Boynton Inlet	Boynton Inlet	ADCP	4 ebb & 4 flood tides	3-4 June 2007
Boynton Intensive-2	Water, Chem & Bio Flux thru Boynton Inlet	Boynton Inlet	ADCP	4 ebb & 4 flood tides	26-28 Sep 2007
Boynton Inlet Flows	1-year measurment of Boynton Inlet flow	Boynton Inlet	ADCP	horizontal ADCP	Feb-07 thru Oct-08
GSR-ADCP	1-year measurement of ocean current at GSR	GSR reef	ADCP	vertical ADCP	Apr-07 thru Jul-08
SC Monitoring Cruises	WQ sampling program around SC outfall	SC outfall	R/V Cable	six bimonthly cruises	June, Aug, Oct 07; Feb, May, Jul 08

Current Measurements. Three in-situ Acoustic Doppler Current Profilers (ADCP, RD Instruments) were installed to measure ambient currents in the area of the SC. A 300-kHz ADCP was installed at the south end of Gulfstream Reef (26°29.272'N, 80°2.35'W, 16.4 m depth, deployed 29-Sep-2006 to 1-Jul-2008). A 600-kHz ADCP was installed at the north end of Gulfstream Reef (26°31.247'N, 80°1.939'W, 14.5m depth, deployed 4-Apr-2006 to 9-Aug-2007).

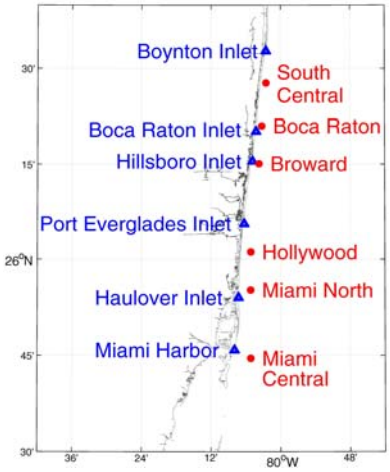

Figure 1. Locations of coastal inlets (triangles) and treated wastewater treatment plant outfalls (circles) in the FACE area of study.

Thirdly, a 1200-kHz ADCP was installed midway between the Boynton Inlet and the north end of Gulfstream Reef (26°32.004'N, 80°2.146'W, 14.8 m depth, deployed 20-Feb-2007 to 13-Apr-2007). These ACDPs yielded ambient current direction and magnitude measurements through the water column from near the ocean bottom to near the ocean surface. They also recorded ambient water temperature and pressure.

Nutrient Measurements. Water samples from bottom, mid, and surface waters at fifteen locations around the SC and the Boynton Inlet (Fig. 2) were obtained on bimonthly cruises for one year (see Table 1) and analyzed for a variety of nutrients, including ammonia (EPA Method 349.0; Zhang et al. 1997a), nitrate + nitrite (EPA Method 353.4; Zhang et al. 1997b), orthophosphate-P (EPA method 365.5; Zhang et al. 2001), total phosphorous (EPA Method 367.0; Zhang et al. 1998), and silicate (EPA Method 366.0; Zhang and Berberian 1997). Analyses were performed on a modified 5-channel Perstop Auto analyzer generally within three hours after sample collection.

Microbial Studies. Water samples collected from all cruises and inlet studies in Table 1 were analyzed for a variety of microbes, including fecal-indicating bacteria (FIB), pathogens, and source tracking markers. Viable enterococci FIB were enumerated using IDEXX Enterolert™ (EPA 2003). *Cryptosporidium* oocysts and *Giardia* cysts (protozoan pathogens) were determined by immunomagnetic separation and immunofluorescent microscopy (EPA 2001). Water samples for molecular analysis of viruses, bacteria, and source

tracking markers (1 L) were processed by membrane filtration prior to nucleic acid extraction. Water samples for analysis of protozoans (>100 L) were processed using FiltaMax™ cartridges (IDEXX). RNA viruses (noroviruses and enteroviruses) were analyzed by real-time quantitative reverse-transcription PCR (qRT-PCR). Standard PCR was used for detection of *Campylobacter jejuni*, *Salmonella* spp., *Escherichia coli* O157:H7, *Staphylococcus aureus*, human-specific enterococci, and adenovirus (LaGier et al. 2004). Real-time PCR (qPCR) was used to quantify enterococci, human-specific *Bacteroides*, and human-specific *Methanobrevibacter smithii*.

Results

Dilution of the outfall plume based on tracers. The tracer study conducted on the Hollywood outfall in 2004 demonstrated the utility of using SF_6 as a tracer for study of the farfield plume (i.e., > 400 m from the outfall). Transportation and dispersion of the plume depended on the physical oceanographic conditions encountered during the time of the study. The plume was incorporated into the ambient ocean current, generally northerly during these experiments, and remained in the upper portion of the water column following the initial rise out of the outfall pipe. The relationship determined for the dilution of treated wastewater (Dilution = 212 x distance [km] from the outfall, Wanninkhof et al. 2005) over 60 km was consistent with earlier outfall studies performed in that area within 2 km (Proni et al. 1994). In the 2007 tracer study, RD results suggested a dilution of approximately 233:1 in the area around the SC boil.

Currents. ADCP data for the 2004 tracer study were collected in the region of Dania Beach, FL. These currents were generally northward with an average flow of about 20 cm/s measured at 1.5 km from shore; although reversals to the south were observed during the six-day period of the study (Wanninkhof et al. 2005). ADCP data were collected near the SC outfall over a longer time frame. As observed in the area of the Hollywood outfall, currents to the south were measured; however, a northern current was measured ~86% of the time. The mean northward current was ~32 cm/s to the north at 3 m depth, tending to decrease with depth below 3 m. In addition, there was a mean eastward component to the current, measured at 6 cm/s at 3 m depth. The eastward component was fairly uniform with depth.

Nutrients. Only a small subset of the nutrient data collected to date is presented here. Averaged (over surface, mid, and deep-water values) nitrate + nitrite ("N+N", Fig. 3) and silicate concentrations (Fig. 4) were elevated in the area of the Boynton Inlet and the Lake Worth lagoon (Fig. 2) relative to surrounding areas, including in comparison to concentrations measured on and around the boil of the SC treated wastewater outfall. Ammonia concentrations (Fig. 5) were sometimes elevated in the lagoon (Oct 2007, July 2008) and sometimes at the boil (Aug 2007, May 2008). The highest average concentrations of orthophosphate-P were measured in the lagoon and inlet (0.54 and 0.2 µM, respectively; n=6 bimonthly cruises). Concentrations were lower at the boil (0.07 µM) and elsewhere (<0.04 µM).

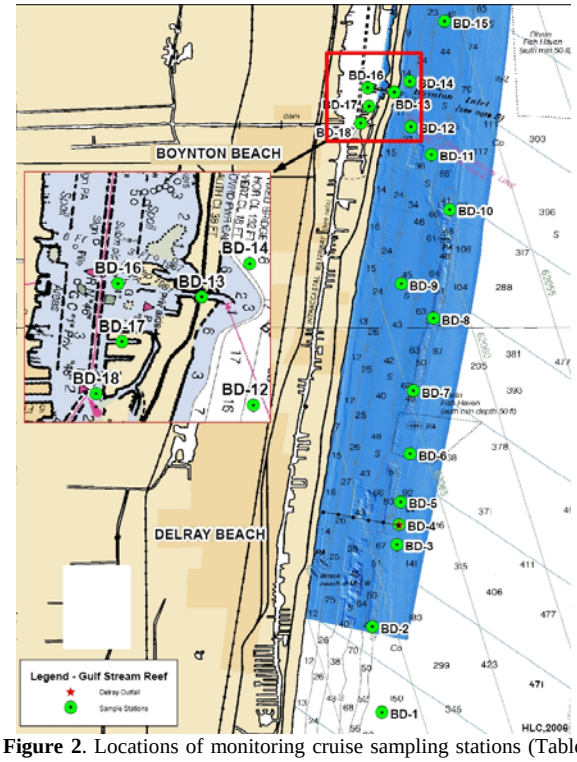

Figure 2. Locations of monitoring cruise sampling stations (Table 1). The SC ocean outfall is at BD-4, the Boynton Inlet at BD-13. Samples from within the Lake Worth lagoon are BD16, 17, and 18.

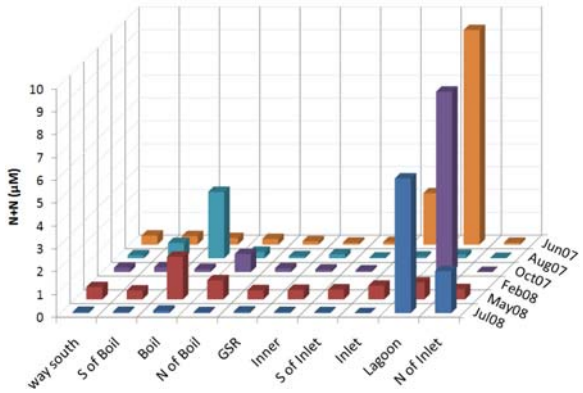

Figure 3. Concentrations of nitrate+nitrite from bi-monthly cruises. Referring to Figure 2, averaged concentrations are given for Way South = BD1 and BD2; S of Boil = BD3; Boil = BD4; N of Boil = BD5; GSR = BD6, BD7, BD8, and BD10; Inner = BD9; S of Inlet = BD11 and BD12; Inlet = BD13; Lagoon = BD16, BD17, and BD18; N of Inlet = BD14 and BD15.

Two 48-hour sampling intensives at the Boynton Inlet examined the nutrient loads from a total of eight ebb tides. A side-looking ADCP provided accurate flow data. A comparison was then made between the nutrient loads from the SC outfall (Koopman et al. 2006) and that from the nearby Boynton Inlet, shown in Table 2. Although there was significant variance in the Inlet ebb tide pulse masses, the average daily loading significantly exceeded or was comparable to that from the SC outfall.

Table 2. Mass loadings (kg/d) on ebb tide pulses from the June and September Boynton Inlet experiments (left) compared to the output of the South Central TWWP (right). For phosphorous, intensive units are dissolved inorganic phosphate; for SC the units are total phosphate (*), which includes particulate phosphate.

	June Intensive				Sept Intensive				Inlet Ave	SC Ave
	1	2	3	4	1	2	3	4	Mass	Mass
N+N kg [N]	204	99	138	23	569	476	303	105	479	191
Si kg [Si]	1196	955	1452	798	5255	4134	2248	1215	4313	
P kg [P]					362	195	116	31	352	79*
NH_4 kg [N]	171	68	89	41	599	524	286	78	464	545

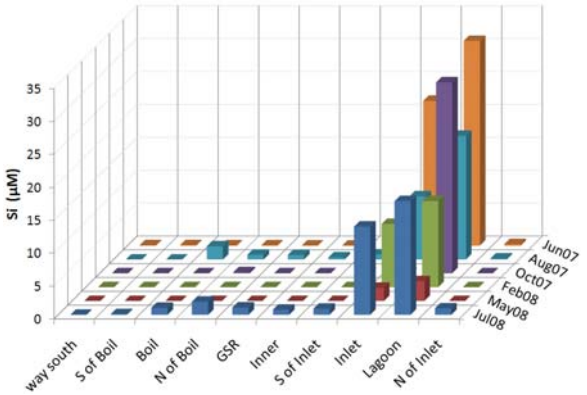

Figure 4. Concentrations of silicate from bi-monthly cruises. Sample denotations are as described in Figure 3.

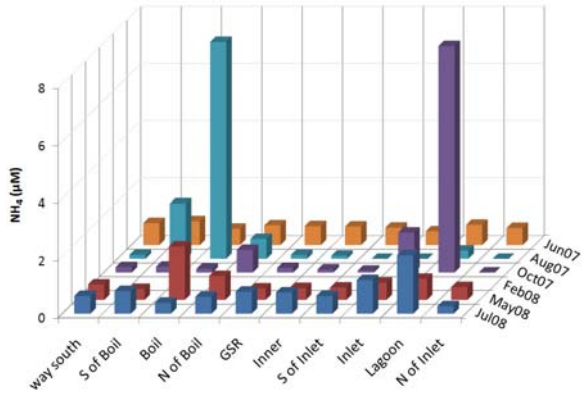

Figure 5. Concentrations of ammonia from bi-monthly cruises. Sample denotations are as Figure 3. No measurements were obtained from the February 2008 samples.

Microbiology. Only a small subset of the data collected to date is presented here. The inlet appeared to be a source of microbial contaminants to near shore waters as indicated by a higher percentage of positive detections for pathogens, FIB, and source tracking markers associated with the outgoing tide versus the ingoing tide (Fig. 6). A variety of microbial contaminants were detected in outgoing tides from the inlet. In comparison, water samples taken from the boil and near the bottom of the SC outfall (the closest outfall (the closest outfall to the Boynton inlet) did not yield positive results in February 2007 (Table 1). A low amount of enterococci DNA (<30 genome equivalents) was detected at the SC boil during a July 2008 cruise, and the abundance declined with distance from the outfall (Fig. 7).

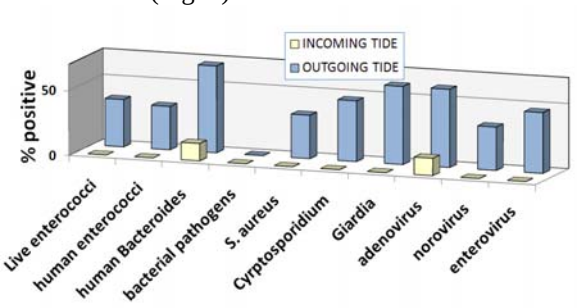

Figure 6. Detection of microbial contaminants for incoming vs. outgoing tides during a 48-hr intensive study at the Boynton inlet. Data show the percentage of samples showing positive detection for microbial contaminants out of 15 discrete time points. "Bacterial pathogens" is a composite for *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli* O157:H7.

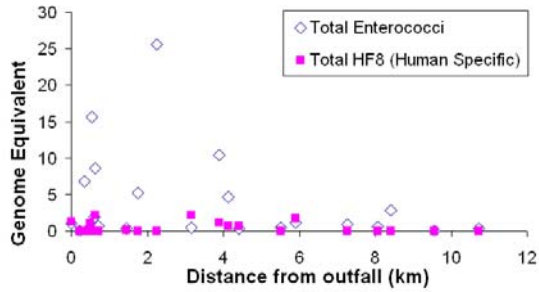

Figure 7. The abundance (genome equivalents) of enterococci and a human-specific *Bacteroides* (HF8) as measured by qPCR versus distance from the SC outfall (data from July 2008 R/V Walton Smith cruise).

Table 3. Microbiological data from surface water and bottom (btm) of the SC wastewater boils from a Feb. 2007 cruise aboard the Coral Reef II. No detection is indicated by "nd".

Target	surface	bottom
Live enterococci	-	-
human *Bacteriodes*	-	-
human enterococci	-	-
adenovirus	-	-
norovirus	-	-
MS2 phage	-	-
enterovirus	-	-
Salmonella ssp.	-	-
S. aureus	-	-
Cryptosporidium/100 L	2	nd
Giardia/100 L	3.4	nd

Compared to the SC outfall, samples from other outfalls yielded higher abundances of *Cryptosporidium* oocysts and *Giardia* cysts (Table 4) In general, the abundance of protozoan cysts appeared to dilute with distance because cysts were not detected at a distance of 1 km from the outfall (data not shown).

The abundance of norovirus and the human-associated FIB, *M. smithii* also were higher at the other outfalls tested (Table 4). These data provide a measure of total abundance; the percentage of viable organisms currently is unknown and is potentially low for these chlorinated effluents discharged into sun-lit coastal waters.

Table 4. Abundance of *Cryptosporidium*, *Giardia*, human-specific FIB, *M. smithii*, and human viral pathogens of the norovirus group measured by qPCR from surface water of wastewater boils from a Feb. 2008 cruise aboard the R/V Nancy Foster. (GE = Genome Equivalents).

Location	*M. smithii* (GE/100ml)	*Norovirus* (GE/100ml)	*Cryptosporidium oocysts*	*Giardia cysts* /100 L
SC boil	700	nd	nd	nd
Hollywood boil	3.0E+05	235	55	67
Boca Raton boil	2.7E+04	2.3	<1	<1
Broward boil	3.7E+04	6.3	8	2
Miami-N boil	1.3E+05	347	236	246
Miami-C boil	3.4E+05	11	8	120
deep water control	nd	nd		

Discussion

The data derived from the FACE work to date indicate that: (1) for the majority of the time the ambient current at the SC outfall vicinity flows to the north (slightly northeast) approximately 86% of the time; (2) minimal downward mixing of the effluent plume at any outfall was observed within the limits of the observations; (3) The water quality measurements made in the vicinity of the SC outfall showed that there was not a significant elevation of nutrient concentrations (N+N, NH_4, P) in samples at the GSR compared to the southernmost samples (in northward current regimes); (4) The flux of nutrients from the Boynton Inlet significantly exceeds (N+N and P) or is on the same order (NH_4) as that of the nearby SC outfall, indicating the importance of inlets as a source of nutrients to the coastal ocean.

In the case of microbial contaminants, the levels of microbial contaminants observed in the immediate vicinity of the southern outfalls and in some outgoing tides of the Boynton Inlet would be of public health concern were they in drinking water or full-body-contact recreational water. However, given the rapid dilution observed, it is likely that infectious risk to humans and fisheries is very low, except potentially in the immediate vicinity of the southern outfall boils.

The FACE project has collected a variety of data, including nutrient, microbiological, and oceanographic information to help understand the processes that effect Florida's coral reef habitat. The data presented here highlight the need to assess the coastal zone in a cohesive way, especially if data will be used to determine the impacts of land-based pollutants, and for the formulation of science-based regulation.

Acknowledgement

Acknowledgement is given to Source Molecular for analysis of *Cryptosporidium* oocysts and *Giardia* cysts. We thank the NOAA Hollings Marine Laboratory, Charleston SC for analysis of *M. smithii* and RNA viruses for selected samples. This research was carried out in part under the auspices of the Cooperative Institute for Marine and Atmospheric Studies (CIMAS), a joint institute of the University of Miami and the National Oceanic and Atmospheric Administration, cooperative agreement #NA17RJ1226.

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Coral Reef Information System (CoRIS): A One Stop Shop for Coral Information

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Abstract. As a coral reef ecosystem manager, one may be overwhelmed with the range of threats encountered on a daily basis. "How do I diagnose coral diseases?" "Which invasive species may inhabit my reef area?" Scientists and students may also find themselves perplexed over the answers to these same questions. "What is being done to combat these issues?" "Where can I find discussions on coral related topics?" The answers to these questions, and many more, may be found in the National Oceanic and Atmospheric Administration's (NOAA's) Web-enabled Coral Reef Information System (CoRIS). CoRIS data discovery tools provide access to metadata, data, and information from the NOAA Coral Reef Conservation Program (CRCP) and other coral reef projects. CoRIS offers original essays that describe coral biology and physiology, reef structure, and types of reefs, among other topics. The CoRIS Library enables searching and browsing through a growing collection of NOAA's coral ecosystems-related publications, reports, Web sites, educational materials, and digital images. One of the most popular features of CoRIS, the Glossary, defines thousands of terms used in coral reef science and management. Whatever your coral reef data and information needs may be, you can begin your search at the CoRIS website.

Key Words: Coral Reef Conservation Program, Metadata, Data.

Introduction

Destructive fishing practices, increases in sea surface temperatures, habitat destruction, diseases, and invasive species: such are the trials and tribulations in the life of a coral reef. The world's coral reefs are threatened and in decline. It is estimated that 10 percent are now beyond recovery, 30 percent are in their critical stages and may die in the next 10-20 years, and 60 percent may die by the year 2050 (USCRTF 2000).

As a coral reef ecosystem manager, one may be overwhelmed with the range of threats encountered on a daily basis. "How do I diagnose coral diseases?" "What are others doing about habitat destruction so that I can learn from their work?" "Which invasive species may inhabit my reef area?" Scientists and students may also find themselves perplexed over the answers to these same questions. "What is being done to combat these issues?" "Where can I find discussions on coral related topics?" The answers to these questions, and many more, may be found in the National Oceanic and Atmospheric Administration's (NOAA's) Web-enabled Coral Reef Information System (CoRIS).

Background

In 1998, the Presidential Executive Order #13089 was issued to preserve and protect U.S. coral reef ecosystems, and the United States Coral Reef Task Force (USCRTF) was established. In turn, NOAA formed the Coral Reef Conservation Program (CRCP) to guide NOAA's coral efforts.

The USCRTF created the National Action Plan to Conserve Coral Reefs, which called for Web-enabled access to Federal agency coral data and information. In response, the CRCP and NOAA's National Oceanographic Data Center developed and maintain the Web-based CoRIS.

CoRIS is designed to provide a single point of access to NOAA data and information for the management and preservation of the United States' coral reefs. The data and information are primarily derived from NOAA's CRCP. Other goals of CoRIS are to meet the information needs of NOAA managers in the preparation of biennial assessments on the status and trends in U.S. coral reef ecosystem conditions, support NOAA's contribution to the USCRTF National Action Plan to Conserve Coral Reefs, and to facilitate archiving and preserving NOAA's coral reef data and information.

Features

CoRIS provides access to over 18,000 coral ecosystem data and data products by way of more than 2,000 metadata records through the "Discover NOAA's Data" section. Users can access the information in several ways:

1) a Google-Maps based search (Fig. 1) which allows users to search for data and information by geographic

area or by specific data types (such as aerial photos, satellite imagery, habitat maps, reef locations, etc.);
2) a Regional Portal search (Fig. 2) which provides access to all available CoRIS data and information by searching specific regions;
3) a text-based search; and,
4) a metadata browse page which provides a list of all available data from the Web site.

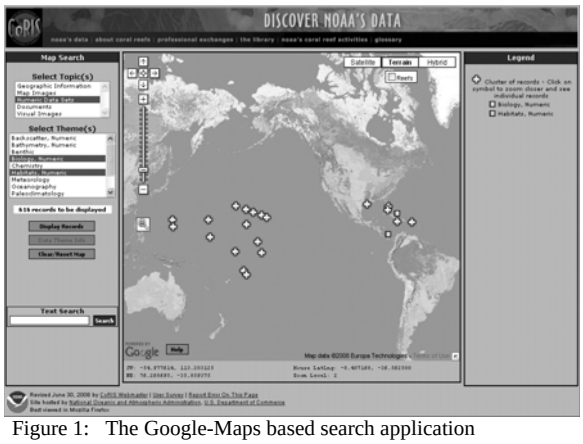

Figure 1: The Google-Maps based search application

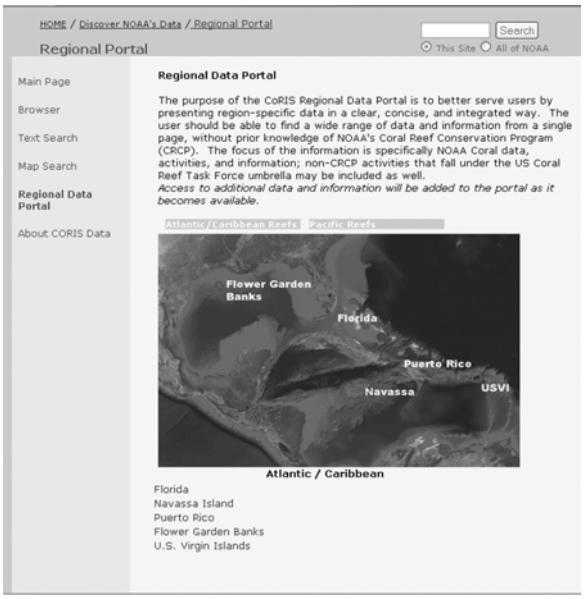

Figure 2: The Regional Portal Search

CoRIS houses a Library collection of over 1,500 publications, reports, journal article citations, Web sites, and more. CoRIS provides direct links to such reports as:

- the "NOAA's Coral Reef Ecosystem Research Plan for Fiscal Years 2007 to 2011" (Puglise and Kelty 2007)
- the "Reef Manager's Guide To Coral Bleaching" (Marshall and Schuttenberg 2006)
- "The State of Deep Coral Ecosystems of the United States: 2007" (Lumsden et al. 2007)
- "The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008" (Waddell and Clarke 2008)

Users can search the Library collection, including NOAA Central Library coral ecosystem publications, through a simple and advanced text search, as well as specific browse lists, for available publication information. There are also direct links to the NOAA Photo Library, and both NOAA and non-NOAA coral related Web sites.

CoRIS also provides access to a collection of coral reef essays on a variety of topics including coral biology, deep water corals, coral diseases, and more. There are also ecosystem essays on areas such as the Northwestern Hawaiian Islands and Navassa, and a collection of professional exchanges on pressing coral topics and issues. CoRIS also has an illustrated glossary containing over 5,000 coral ecosystem related terms, as well as information and links to other coral reef activities throughout NOAA.

Some of the types of data and information available from CoRIS include:

- *in situ* biological, chemical, geological, and physical environmental data collected by divers, remotely operated vehicles, moored buoys, current meters, and other types of oceanographic instruments
- Advanced Very High Resolution Radiometer sea surface temperature products and other remotely sensed imagery products
- coastal aerial photographs
- paleoclimatology data derived from coral core analyses
- nautical charts and tidal data
- coastal bathymetry
- digital video and photographs
- benthic habitat maps
- coral bleaching reports

All of the CoRIS search engines rely on metadata files that describe collections of data sets and products. Metadata provide descriptions of the data, when and where the data were collected, who collected the data, direct links to the data when available, and how to obtain a copy of it. Metadata descriptions use the format for the Content Standard for Digital Geospatial Metadata set by the Federal Geographic Data Committee (FGDC 1998), as well as the standards of the National Biological Information Infrastructure for descriptions of biological data sets containing taxonomic names for organisms (FGDC and USGS 1999). To support the data and information discovery process, CoRIS uses a set of standardized keywords to describe thematic topics, geographic areas, and taxonomic names of organisms.

Conclusion

The NOAA Coral Reef Information System provides access to coral reef ecosystem data and information through a variety of discovery and access tools in order to help managers, researchers, students, and others to better protect and preserve the world's coral reef ecosystems. CoRIS has a wealth of data, information, and other resources to offer. Whatever your coral reef data and information needs may be, you can begin your search at the CoRIS website (CoRIS 2008).

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Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008
Session number 16

Biological and physical characteristics of a mesophotic coral reef: Black Jack reef, Vieques, Puerto Rico

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Abstract. For six decades Vieques Island was used for US Navy military activities. While several studies have addressed the possible impact on shallow coral reefs, mesophotic reefs (30-100 m deep) have been largely overlooked. This study provides an extensive baseline qualitative and quantitative characterization of Black Jack Reef including variations in the light attenuation coefficients, which serve as a proxy for changes in water quality. Operational limitations due to depth were solved using the Seabed Autonomous Underwater Vehicle (AUV). This AUV specifically designed for benthic surveys, was used to obtain two perpendicular phototransects covering a total of 5km at depths ranging 30-51 m. Mean live coral cover was 32% with *Montastraea* and *Agaricia* as the dominant genera. Very low levels of bleaching or diseases were found. Sponges were present in both transects with an average cover of 7-9%. The most common sponges were *Amphimedon compressa, Aiolochroia crassa, Agelas*, *Aplysina* and *Xestospongia muta*. Gorgonians and Black corals were present in very low abundance. Extensive areas of rhodolith beds were found. Turbidity does not appear to be a determinant factor at Black Jack Reef.

Key words: mesophotic reefs, Seabed AUV, Vieques

Introduction

The islands of Vieques and Culebra are in the northeastern Caribbean within the Puerto Rico- Virgin Island geological platform. Culebra and Vieques were used for US Navy military activities for many decades. Military activity at Vieques began in the 1940's and has just recently ended with the hand over of military owned lands to the Department of the Interior in May of 2003, becoming part of the Vieques National Wildlife Refuge. Vieques coral reefs are considered among the best in Puerto Rico in terms of live coral cover (greater than 30%) (García-Saís et al. 2001, 2004).

During the military occupation much of its area was closed to the public and little scientific research was permitted. Ecosystem damage has long been recognized by Rogers et al. (1978) and military debris can still be seen today (Riegl et al. 2008). However, decline in coral cover has been attributed to hurricanes (Antonius and Weiner 1982), sea surface temperature (Dodge 1981), turbidity (Deslarzes et al. 2006) or more recently the combination of hurricanes and diseases (Riegl et al. 2008). Riegl et al. 2008 concluded that the impact of bombing in the coral reef was negligible and, even more, suggested that the military occupation prevented further damage derived from land-based development.

In 2001 García-Saís et al. performed a baseline study outside the shooting range followed by monitoring in 2004. Deslarzes et al. (2006) compared Vieques and Culebra concluding that Vieques reefs were in worst condition. Bombing activities in Culebra stopped in 1975. Riegl et al. (2008) studied the geomorphology and coral assemblage structure and found that Vieques reefs are comparable to those of St. Croix, US Virgin Islands. Recent reef mapping efforts in these areas have been carried out by Hernandez-Cruz et al. (2006), Kendall and Eschelbach (2006), and Monaco et al. (2001). Although shallow reefs (upto 15 m depth) have received much attention, especially within the US Navy shooting range, no studies have taken deep reefs into account. Deeper reefs are typically unaffected by hurricanes or high sea surface temperatures (Riegl and Piller 2003; West and Salm 2003). Practically all mesophotic (30-100 m) coral reef communities of Vieques have not been properly characterized (García-Saís et al. 2008). The only exception to this has been Black Jack Reef (30-40 m depth), which was described in 2001 and characterized in 2004 using five 10 m transects (García-Saís et al. 2004). Further exploratory surveying, mapping, and monitoring of Vieques deep coral reef systems is needed (García-Saís et al. 2008). On the other hand, none of the previous studies has detailed the sponge species found considering they could be an important reef component.

The transparency of the surrounding waters and the relatively wide insular platform of Vieques create the potential for numerous mesophotic reefs, especially in

the south. However, operational limitations due to depth require the use of new technologies for fast and safe assessment and monitoring. Satellite and airborne techniques are limited to the first 20 m and submersibles are generally too costly for most monitoring programs. The Seabed Autonomous Underwater Vehicle (AUV) was specifically designed for benthic surveys up to 2000m deep. It has proved succesful in previous studies in southwestern Puerto Rico (Armstrong et al. 2002; Singh et al. 2004) and in the Hind Bank Marine Conservation District (MCD), USVI (Armstrong et al. 2006).

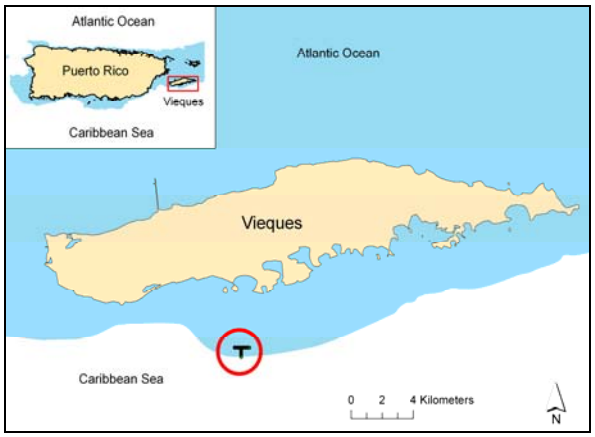

Figure 1. General location of Vieques and transects. The red circle south of the island shows relative position of the two perpendicular transects. Blue shade corresponds to the insular platform.

The aim of this work is: 1) to provide a more extensive (5 km) baseline description of mesophotic reef areas in Black Jack Reef, southern Vieques (Fig.1), 2) to generate a qualitative and quantitative evaluation of coral and sponge taxa, and 3) to describe patterns of light attenuation variation as a proxy for changes in water quality parameters.

Material and Methods

The Seabed AUV is composed of two cylindrical body sections fixed together, coupled with three thrusters and equipped with: a Pixelfly 1024 x 1280 pixel resolution CCD camera with 12 bits of dynamic range, a 150 Ws strobe, a paroscientific depth sensor and a 300 kHz Acoustic Doppler Current Profiler (ADCP). This AUV was designed to be hover capable and to maintain a constant direction and altitude from the bottom. More information on Seabed components and sensors, control and navigational systems can be found in Singh et al. (2004).

During November 2004 we used the Seabed AUV to obtain two long phototransects at Black Jack Reef in southern Vieques Island (based on García-Saís et al. 2004) covering a total of 5 km at depths ranging from 30 to 51 m.

The AUV digital images were analyzed using Coral Point Count with Excel extensions (CPCE, Kohler and Gill 2006). Five major benthic groups were considered: scleractinians, sponges, black corals (Antipatharians), algae and gorgonians. Benthic organisms were identified to the lowest possible taxon and special attention was given to scleractinian and sponge species as well as type of substrate. Coral cover was estimated using the random point method. Since there were large areas of unconsolidated sediment with encrusting algae and cyanobacteria as well as large extensions of rhodolith beds and our main interest was focused on patterns of distribution of benthic mega-invertebrates, bare substrate, algae and cyanobacteria were grouped under the general category: SUBSTRATE+ALGAE+CB (see Figs. 2, 3).

Temporal variation in the light attenuation coefficient (K490) was obtained from MODIS Aqua level 3 imagery at 4 km resolution, producing monthly averages from 2003 to 2008.

During April 2008, salinity, temperature and light attenuation profiles were measured in Vieques using a Satlantic Hyperpro spectroradiometer.

Results

Two phototransects from southern Vieques were analyzed: transect 1 was 1 km long and oriented from north to south (starting at 18.0507N, 65.4633W), while transect 2 was 4 km long and oriented east to west (18.0558N, 65.4586W). A total of 894 and 1540 images, respectively were produced at a constant altitude of 2.5 m from the bottom. Depth ranges were 30-51 m for the North-South transect and 34-43 m for the West-East transect.

The North-South transect (Fig. 2) was composed of about 30% coral reef (defined as areas with a certain structural complexity and some coral cover) and the other two thirds consisted of an extensive rhodolith bed with a few sponges, gorgonians and corals sparsely distributed. The West-East transect (Fig. 3), however consisted of 75% coral reef with 60% coral cover in some areas at 36 m depth. Only the most western part had sand or fine sediment (data not shown). No military debris was identified in any of the phototransects. A substantial amount of rubble and bare substrate could be seen at the southern part of the North-South transect.

At least 36 species of sponges and 17 species of corals were identified. The *Montastraea annularis* species complex, *M. cavernosa* and *Agaricia* spp. were the most common corals found. The most common sponges were *Amphimedon compressa, Aiolochroia crassa, Agelas* sp., *Aplysina* sp. The barrel sponge (*Xestospongia muta*) was also abundant.

Mean live coral cover at reefal sites was lower in the North-South transect (3-11%) than in the West-East transect (10-48%) (Figs. 2 and 3, respectively). In the North-South transect (Fig. 2), the non-reefal part of the transect was composed of a rhodolith bed (98% cover), hardly any macroalgae, some sparse sponges (2% cover) and corals (less than 1% cover). Gorgonian cover was quite low (6 % cover) at reefal sites, and was nearly zero in the non-reefal zone. Black coral cover was less than 1% in both transects.

The West-East transect (Fig. 3), shows remarkable high average mean coral cover at the end of the transect (48%) with an average of 25% live coral cover for the entire 4 km transect. Algae and bare substrate covered an average of 64% of the substrate. Sponges had an average cover of 9%, reaching 20% for the western part of the transect. Gorgonian cover however was less than 1% throughout the entire transect. The westernmost part of the transect consisted of large areas of sand/sediment accumulation.

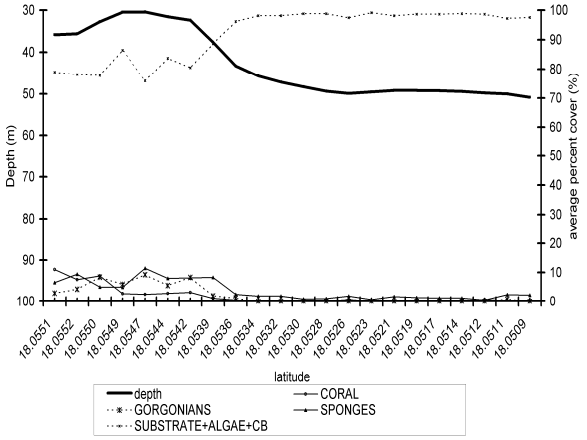

Figure 2. North-South transect average percent cover of each category (obtained every ten frames).

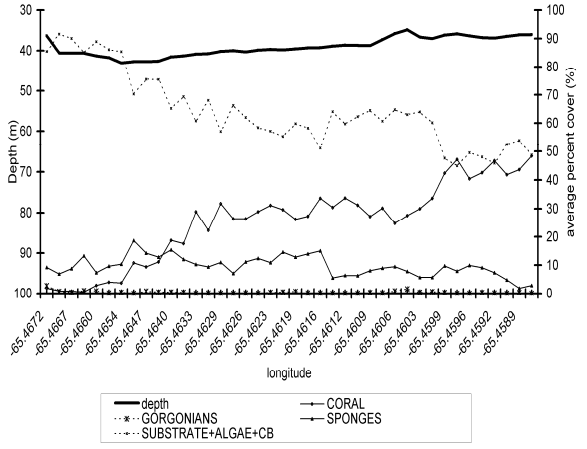

Figure 3. West-East transect average percent cover of each category (obtained every ten frames).

The relationship between coral cover and depth show a negative correlation for both transects (Fig.4, 5), indicating that coral cover decreases with increasing depth. The relationship (R^2) is stronger for the West-East transect (0.33 vs. 0.25).

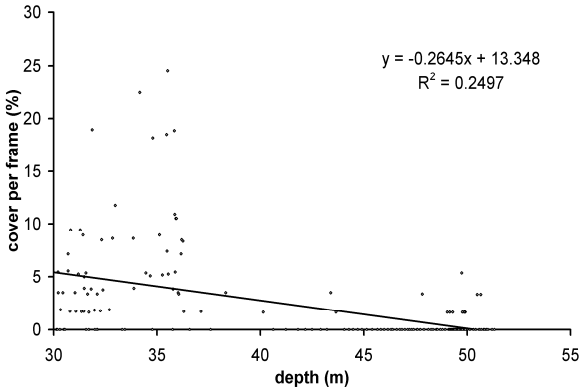

Figure 4. Live coral cover percentage per frame vs. depth in the North-South transect.

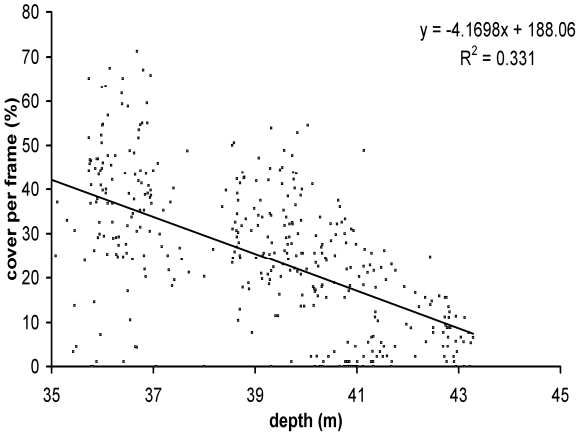

Figure 5. Live coral cover per frame vs. depth in West-East transect.

Profiles of temperature and salinity, which were measured every meter from the surface to 45m depth, ranged from 25.8-26.2 ºC, and 37-38.5 psu, respectively. The Ed PAR (downwelling irradiance) profile (Fig. 6) demonstrates the transparency of these waters with 10% of the surface Ed present at 39 m. The surface Ed is approximately 1500 $\mu W/cm^2/nm$. Satellite derived K490 show little variation from 2003 to 2008, with average values ranging from 0.03 to 0.04 m^{-1} (Fig. 8). Higher values were present in July 2006 (over 0.05 m^{-1}) whereas April 2007 had particularly low values at 0.02 m^{-1}.

Southern Vieques waters are very clear throughout the year although they become slightly more turbid during the rainy season (July to December). Gaps in June are due to high cloud cover in the satellite images.

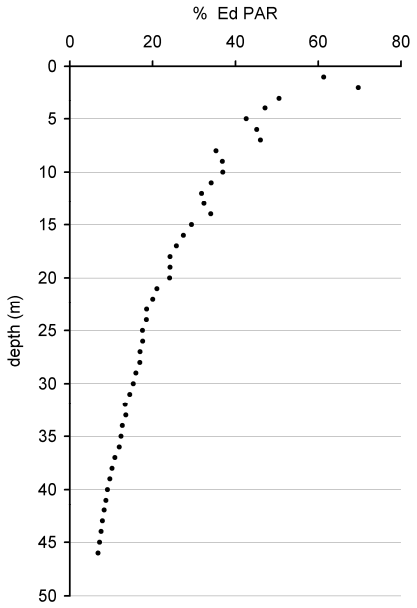

Figure 6. Light attenuation with depth. Percentage of Photosynthetically Available Radiation (PAR 400-700nm) per depth in South Vieques during April 2008.

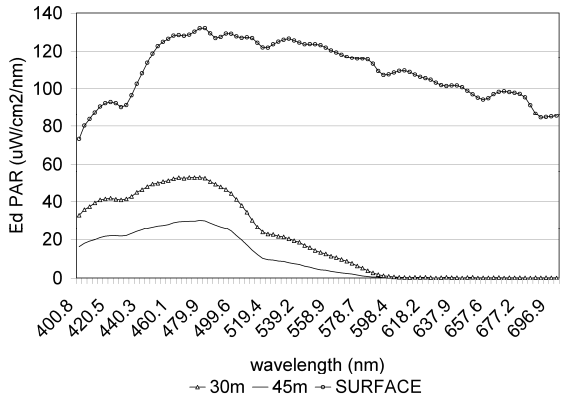

Figure 7. Downwelling PAR irradiance spectra at surface, 30 and 45 meters deep in South Vieques during April 2008.

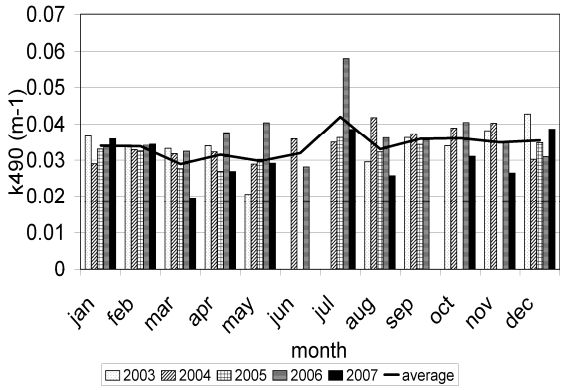

Figure 8. Temporal variation of light attenuation coefficient at 490 nm (K490). Average value per month per year from 2003-2008 calculated using MODIS Aqua data south of Vieques at 18.05N, 65.46W.

Discussion

The two AUV transects provided detailed information on benthic cover and geomorphology of a Vieques mesophotic coral reef complex. In most cases, the AUV imagery resolution was adequate to identify organisms to at least genus level if not species level, for both corals and sponges as well as other non-motile mega-invertebrates. These mesophotic reefs appear not to be as extensive as those described for the Hind Bank MCD (17 km east of Vieques) by Armstrong et al. (2006) with mean live coral cover of 32% as opposed to 43%, respectively. Coral cover values are similar to the 28.8% reported at Black Jack Reef by García-Saís et al. (2004). In all three cases, *Montastraea annularis* species complex dominated live coral cover.

Sponges were continuously present in both transects with an average cover of 7-9% and up to a maximum of 26.9% at the western part of West-East transect at a depth of 43 m. Average sponge cover values are similar but slightly higher than the 5.8% reported at Black Jack Reef by García-Saís et al. (2004) and 3.8% at the Hind Bank MCD (Armstrong et al. 2006). *Cliona* was not the dominant species as in the MCD where it accounted for 96% of the sponges found (Armstrong et al. 2006). The morphology of the most frequent sponges found was usually tube or rope-like, except for the massive *Xestospongia muta*, and a few encrusting or spherical-forms.

Gorgonians showed very low cover in both transects, similar to the Hind Bank MCD (Armstrong et al. 2006) and to what was reported at Black Jack Reef by García-Saís et al. (2004). Black corals were present in very low abundance (less than 1%) coinciding with García-Saís et al. (2004).

The distribution of live coral cover appeared to be related to the geomorphology and weakly related to depth. Coral cover was higher in areas of topographic relief. However, sponges were also present over unconsolidated sediments due to their faster growth rates. This pattern was more evident in West-East transect where the western side had an elevated dense coral reef (up to 50% live coral cover) whereas the eastern side accumulated sand and had sparse coral colonies and sponges.

Very low levels of coral disease or bleaching could be seen in the AUV images. This agrees with Armstrong et al. (2006) results from similar depths at the MCD.

Water quality, at least in terms of turbidity, does not appear to be a determinant factor at Black Jack Reef, contrary to what Deslarzes et al. (2006) suggested for Vieques reefs. This is to be expected since Black Jack Reef is deeper and farther from the coast. As in the MCD, most corals presented a plate

shape in response to the low-light environment at these depths. As in shallow water reefs, increases in light attenuation could serve as a precursor of change in mesophotic reefs, where incident irradiance levels could be near the lower limit for coral growth.

Since Black Jack Reef is outside the bombing range, military debris was not expected and was not observed in the AUV imagery. Additional transects within the bombing range will be required to ascertain the possible impact of past military activities in both shallow and deep reef areas.

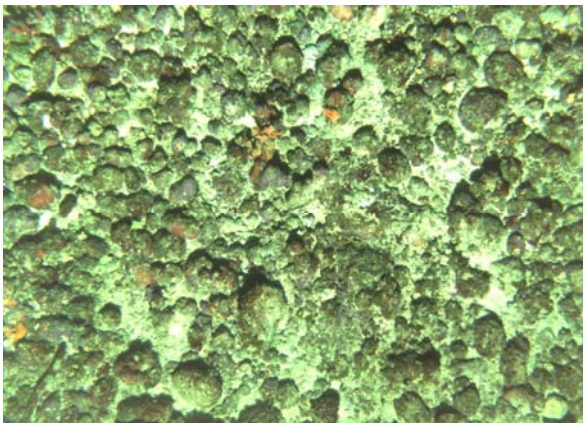

Figure 9. Rhodolith beds found at the end of the North-South transect.

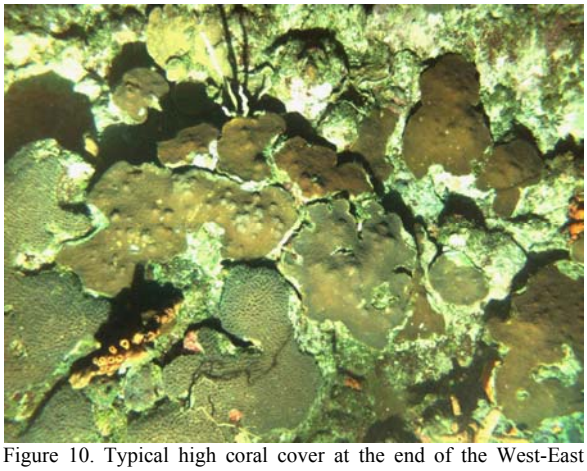

Figure 10. Typical high coral cover at the end of the West-East Transect.

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Colored Dissolved Organic Material Increases Resiliency of Coral Reefs by Controlling Exposure to UVR

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Abstract. Although mass coral bleaching events are generally triggered by high seawater temperatures, experiments have demonstrated that corals and reef-dwelling foraminifers bleach more readily when exposed to high energy, short wavelength solar radiation (blue, violet and ultraviolet [UVR]: λ ~ 280 - 490 nm). In seawater, colored dissolved organic matter (CDOM), also called gelbstoff, preferentially absorbs these shorter wavelengths, which consequently bleach and degrade the CDOM. Alteration and destruction of watershed and coastal wetlands have reduced natural sources of CDOM that are tidally flushed into reefal waters. We have measured absorption of UVR and UV irradiance at various reefs in the Florida Keys that differ in distance from shore and the degree of anthropogenic development of the adjacent shoreline. Our results show that reefs associated with intact shorelines tend to be exposed to lower intensities of UVR than reefs associated with developed shorelines. Absorption due to CDOM at 320 nm (a_{g320}) was less variable at reefs associated with intact shorelines, and higher at inshore reefs compared to offshore reefs. UVR is attenuated more quickly at inshore than offshore, clear-water reefs at similar depths. Spectral slope of a_g, S, was generally greater at offshore sites, indicating a higher degree of photobleaching of CDOM.

Keywords: ultraviolet radiation, coral reefs, absorption coefficient, attenuation coefficient, CDOM

Introduction

Corals worldwide have been declining since the 1970's and the prognosis for the future is not improving (Birkeland 2004; Hoegh-Guldberg et al. 2007). Coral bleaching has become a worldwide phenomenon, and the frequency and intensity of bleaching is increasing (Hoegh-Guldberg 1999; Wilkinson 2002). While the relationship between coral mass-bleaching events and elevated sea-surface temperature (SST) is well established (Hoegh-Guldberg 1999), increasing numbers of studies are revealing that light plays a vital role in coral bleaching. For example, Lesser and Farrell (2004) found that corals do not bleach in the absence of light. Low wavelength ultraviolet radiation (UVR) and blue light can stimulate production of reactive oxygen species causing gene mutation and other damaging consequences to marine invertebrates (Lesser 2006; Levy et al. 2006).

Mass bleaching events typically occur when sea conditions are unusually calm (e.g., Fabricius et al. 2004) and thermal bleaching appears to be caused by photoinhibition and photodamage to photosystem II of the zooxanthellae (e.g., Lesser and Farrell 2004; Smith et al. 2005). The fact that clouds or direct shading can reduce bleaching in corals provides more evidence for the necessary role of light (e.g., Mumby et al. 2001; Fabricius et al. 2004). More recent studies are linking coral disease and photooxidative stress (Lesser 2006).

UVR specifically has been shown to cause DNA damage, DNA mutations or cell death in marine organisms such as corals (Shick et al. 1996). Although it is generally thought that UVR attenuates quickly, some natural water bodies are characterized by high transparency to UVR (Gleason and Wellington 1993).

Pure water absorbs minimally at wavelengths below 490 nm, thus attenuation of the shorter wavelengths of light is primarily due to dissolved and particulate matter (Kirk 1996). Light absorption by colored dissolved organic matter (CDOM) is highest at the shortest wavelengths and exponentially decreases with increasing wavelength. Moreover, the absorption of high-energy radiation causes bleaching and degradation of CDOM (Zepp et al. 2008). Spectrally, photobleaching of CDOM increases with decreasing wavelength from 500 to 280 nm, with the most effective photobleaching occurring in the UV-A region (320 – 400 nm) (Osburn et al. 2001). While an increase in rates of CDOM breakdown may not be biologically significant in turbid, CDOM-rich waters, it may be a major reason why corals in clear waters are reportedly more susceptible to bleaching (e.g.,

West and Salm 2003) and possibly also to diseases that are not directly related to pollution. For example, as a consequence of the 4% global reduction in stratospheric ozone following the Mt. Pinatubo eruption (Randel et al. 1995), the resultant approximately 8% increase in UV-B reaching the sea surface (Schick et al. 1996) could have increased the rate of CDOM degradation by as much as 24% (Zepp et al. 2003). Other studies estimate a lower percentage change, for example, a 25% increase in UV-B results in a 10% increase in photobleaching according to studies on temperate lakes (Osburn et al. 2001).

As a defense against UVR, corals and other aquatic organisms produce UV-absorbing pigments called mycosporine-like amino acids (MAAs) (Shick et al. 1996). Maximum absorption for MAAs occurs between 305 and 360 nm. Thus, the presence of MAAs can indicate photic stress (Morrison and Nelson 2004).

Exposure to UVR has been increasing in recent decades due to stratospheric ozone depletion, resulting in increased photobleaching of CDOM and, in turn, deleterious effects on marine biota (Fig. 1). We postulate that CDOM is protecting inshore patch reefs from exposure to the most extreme solar radiation and damaging effects of photooxidative stress.

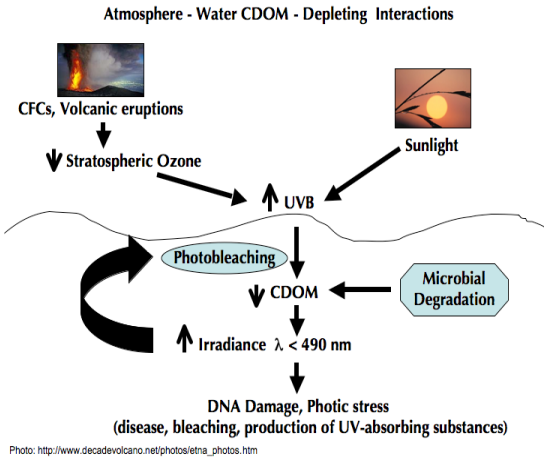

Figure 1. Atmospheric, optical, and biological factors affecting CDOM absorptivity and related biological effects (after Morris and Hargreaves 1997; Zepp et al. 2008).

Material and Methods

In late May, early July and late September 2004, and early May and mid-July 2005, water samples and *in situ* optical data were collected at several reefs in the upper and middle Florida Keys. In addition, in September 2004, absorption due to CDOM (a_g) was measured along a transect (red arrow in Fig. 2) from offshore at 75 m depth, shoreward to 50 m and 25 m depths, inshore to Carysfort and Algae Reefs, and finally within a mangrove-lined canal in John Pennekamp State Park. In summer 2006 and 2007, sampling sites included inshore and offshore coral reefs in the Upper, Middle and Lower Keys that lie within the Florida Keys National Marine Sanctuary (FKNMS) and are part of the Coral Reef Evaluation and Monitoring Program (CREMP) (Fig. 2).

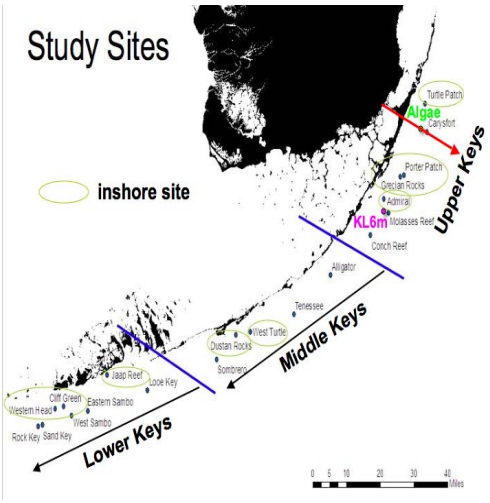

Figure 2. Study sites in the Lower, Middle and Upper Florida Keys included offshore reefs and inshore (patch) reefs that differ in degree of development of associated shoreline. In 2006 and 2007, study sites also included inshore and offshore CREMP study sites in the Lower, Middle and Upper Florida Keys (image collaboration with A. Ramirez).

Total absorption can be partitioned into absorption due to dissolved material, a_{CDOM}, particulate material, a_{PM}, and pure water, a_w (Kirk 1996). Using measured a_{CDOM} and a_{PM}, and published values of a_w (Morel et al. 2007):

$$a_t(\lambda) = a_{CDOM} + a_{PM} + a_w \quad (1)$$

In natural systems, light is not collimated but diffuse. Measuring irradiance consistently within 2 hours of solar noon minimizes the effect of sun angle and thus pathlength on light attenuation. Total attenuation is due to scattering as well as absorption. Scattering is negligible compared to absorption for this study (Ivey, unpubl. data). Thus, the diffuse attenuation coefficient of downwelling irradiance (K_d) can be estimated from total absorption (Kirk 1996):

$$K_d = a_{CDOM} + a_{PM} + a_w \quad (2)$$

Water samples were collected from the subsurface (~ 0.5 m) and at the depth of coral growth by SCUBA divers or using Niskin bottles. After filtration of water samples, samples were frozen and transported back to the lab, where spectral absorption (300-800 nm) for CDOM ($a_g(\lambda)$) was measured according to the method described in Mueller and Fargion (2002) and $a_{PM}(\lambda)$ was measured according to Mitchell (1990) using a UV-Visible spectrophotometer (Perkin Elmer Lambda 18 or Hitachi U 3300). Spectral absorption due to detritus,

a_d (λ), was determined by methanol extraction of pigments and subtracted from $a_{PM}(\lambda)$ to determine spectral absorption due to phytoplankton, a_{phi} (λ):

$$a_{phi}(\lambda) = a_{PM}(\lambda) - a_d(\lambda) \quad (3)$$

(Kirk 1996). Relative MAA expression was determined using the method of Morrison and Nelson (2004).

In July 2004, incident solar irradiance reaching the sea surface was measured using a LiCor - 1800 Spectroradiometer (280-850 nm) at a nearby land site at 10-20 minute intervals from 8:30 am – 6:30 pm daily. Intensity of irradiance reaching the benthos was calculated from measurements of in-lab absorption a_t (λ) and the *in situ* incident downwelling irradiance E_{d0} (λ) according to eqn. (3) and:

$$E_{dz}(\lambda) = E_{d0}(\lambda) * e^{-Kd(\lambda)* z} \quad (4)$$

where z represents depth in meters (Kirk 1996). After July 2005, K_d was calculated from *in situ* underwater downwelling cosine irradiance ($E_d(\lambda)$) measured at 305, 330, 380 nm (10 nm wavebands, recorded at maximum wavelength minus 5 nm) and PAR (integrated from 400 – 700 nm) using a BIC (Biospherical Instruments, Inc.) radiometer.

Differences in a_g, K_d, spectral slope S, and relative MAA expression were determined using one-way ANOVA statistical tests.

Results

Absorption due to CDOM decreased going offshore from mangroves to inshore reefs, offshore reefs and finally blue water (Fig. 3), exhibiting the progressive dilution of land-sourced CDOM.

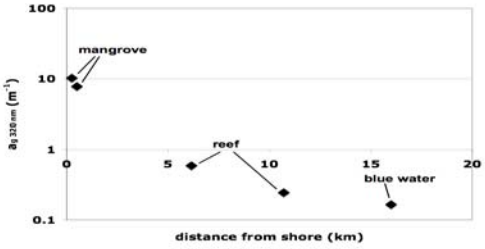

Figure 3. Transect of absorption due to CDOM at 320 an ($a_{g\,320}$). $a_{g\,320}$ decreased going from mangrove canals in John Pennekamp Park to inshore and offshore reefs to offshore blue water.

Downwelling UV irradiance at 320 nm at depth = 6m ($E_{d6m\ 320nm}$), modeled from a_{t320} and incident irradiance (E_{d0}), was significantly higher at reefs associated with developed shoreline, such as KL6m ($E_{d6m\ 320nm}$ = 0.01 - 0.084 W/m^2) than at reefs offshore from extensive mangrove shoreline, such as Algae Reef ($E_{d\ 320nm}$ = 0.008 – 0.057 W/m^2) ($p < 0.05$, Fig. 4).

Over the period of sampling, 2004 – 2007, the average contribution of absorption due to CDOM, a_g, to total absorption, a_t, increased with decreasing wavelength, ranging from 60% at 380 nm to over 90% at 305 nm. Thus, CDOM is the major attenuator of UVR. Over the course of each summer a_g/a_t typically decreased, likely due to photobleaching of CDOM (Fig. 5). The observed increase in a_g/a_t from May to July 2005 may be due to the higher rainfall which occurred in June and July, causing greater runoff and thus increased CDOM over the reef (http://www.ncdc.noaa.gov/oa/climate/research/monitoring.html#ustempprcp).

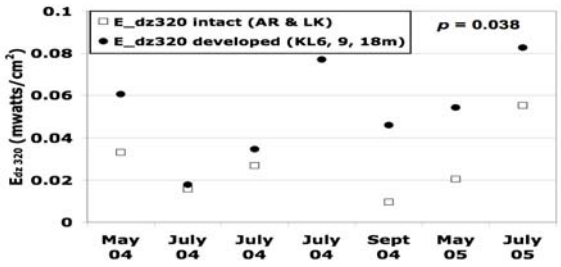

Figure 4. $E_{d6m\ 320}$ was significantly lower at intact shoreline-associated reefs compared to developed shoreline-associated reefs ($p < 0.05$).

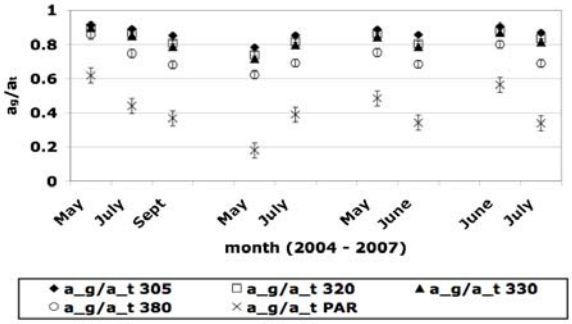

Figure 5. Average relative contribution of a_g to a_t in the UV at 305, 320, 330, 380 nm ranged from 62% at 380 nm to 91% at 305 nm and from 18 – 62 % for PAR. Average a_g/a_t typically declined at all wavelengths as the summer progressed.

Comparing a_g between inshore and offshore reefs, a_g was significantly higher at inshore reefs at all wavelengths (e.g., a_{g320}, Fig. 6, p = 0.0086). Concurrently, K_d measured *in situ* using a BIC radiometer was significantly higher at inshore reefs (ex: $K_{d\ 330}$, Fig. 6, Table 1, p = 0.00297). Differences in spectral quality were also observed: excepting discrepancy from this trend at 305 nm due to immeasurably low irradiance intensities, the difference in K_d between inshore and offshore reefs decreased with increasing wavelength, with the p value for $K_{d\ PAR}$ an order of magnitude higher than for $K_{d\ UV}$ (Table 1). Thus, difference in water transparency between inshore and offshore reefs was greater for UVR than for PAR.

Both a_g and K_d, two independent measures of UV transparency, illustrate that coral reef biota are exposed to lower intensities of UV irradiance at

inshore reefs compared to offshore reefs in the Florida Keys.

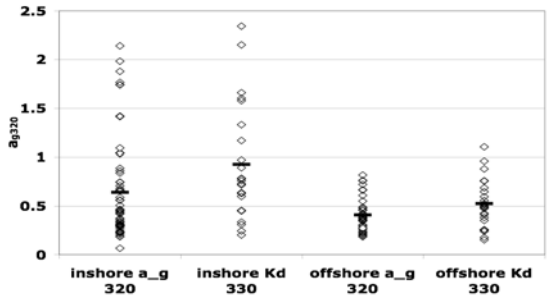

Figure 6. Absorption due to CDOM at 320 nm ($a_{g\ 320}$) was significantly higher at inshore reefs (n = 58, average = 0.64) than offshore reefs (n = 38, average = 0.41) (p < 0.01). The attenuation coefficient of downwelling irradiance at 330 nm, $K_{d\ 330}$, was significantly higher at inshore reefs (n = 23, average = 0.928) than offshore reefs (n = 25, average = 0.524) (p < 0.005). Dashes represent averages.

	average		range		std dev		
K_d (λ)	in	off	in	off	in	off	p
$K_{d\ 305}$	1.40	0.83	0.32 – 1.30	0.27 – 1.83	0.93	0.38	0.008
$K_{d\ 330}$	0.93	0.52	0.20 – 2.15	0.24 – 1.10	0.59	0.24	0.003
$K_{d\ 380}$	0.42	0.25	0.13 – 0.90	0.066 – 0. 56	0.25	0.12	0.003
$K_{d\ PAR}$	0.23	0.18	0.07 - 0.32	0.025 – 0.49	0.06	0.09	0.039

Table 1. Statistical data comparing K_d between inshore and offshore reefs. The difference in K_d between inshore and offshore reefs was greatest for 330 and 380 nm, and least for PAR (in = inshore, off = offshore).

Relative expression of MAAs varied inversely with $a_{g\ 320}$ (Fig. 7) and was higher and more variable at reefs associated with developed shoreline (a_{phi_325} /a_{phi_350} = 1.3 – 2.7, CV = 30%) than at reefs associated with intact shoreline (a_{phi_325} /a_{phi_350} range = 1.03 to 1.2, CV = 6%), (p < 0.05).

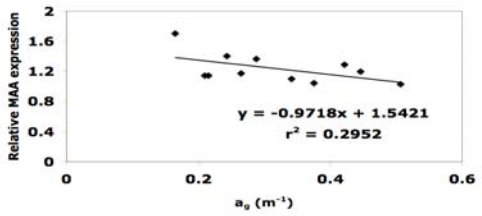

Figure 7. Relative expression of MAAs varied inversely with $a_{g\ 320}$ (slope = -0.97) for intact and developed reefs in 2004 – 2005.

Spectral slope of a_g in the UV, $S_{(280\ –\ 312\ nm)}$, was significantly higher at offshore sites (avg. = 0.030, std. dev. = 0.003) compared to inshore sites (avg. = 0.027, std dev. = 0.004) (p = 0.001). S is an indicator of photobleaching of CDOM (Del Vecchio and Blough 2002). Thus, in addition to higher exposure to UVR (Fig. 6), CDOM at offshore reefs was more highly photobleached.

Discussion

Though traditionally it has been thought that corals require clear water for photosynthesis, recent trends show that the clearer water reefs are experiencing higher rates of coral decline. In the Florida Keys, distance from shoreline as well as shoreline quality may influence reef health as recent declines in percent coral cover and coral biodiversity have been greater at offshore reefs than inshore reefs (Somerfield et al. 2008) and coral-lesion recovery rates are higher at inshore (patch) reefs near intact mangrove shoreline than developed shoreline (Fisher et al. 2007). Inshore reefs may be closer to seagrass beds, mangroves, wetlands, and other terrestrial sources of CDOM. Our work shows that differences in water transparency, and the resulting spectral differences in solar radiation reaching the benthos, may contribute to different rates of decline in coral cover between inshore patch reefs and offshore shallow reefs.

This study helps to support/explain previous observations in the Florida Keys: 1) lower rates of decline at inshore reefs than offshore reefs (e.g., Somerfield et al. 2008); 2) consistently higher bleaching in larger foraminifers at a reef associated with clearer water (Conch Reef) than at a reef influenced by Florida Bay water (Tennessee Reef) (Williams 2002); 3) occurrence of bleaching in benthic foraminifers (*Amphistegina gibbosa*) in the Florida Keys follows solar cycle, not SST cycle, and increases with increasing UV:PAR (Williams 2002); and 4) higher coral cover, coral-lesion recovery rates and abundances of larger foraminifers at a reef associated with intact shoreline (Algae Reef) compared to a reef associated with developed shoreline (KL6m Reef) (Fisher et al. 2007).

Prior studies also show deleterious effects of UVR on reef organisms. Lab experiments have shown that bleaching in *A. gibbosa* is exacerbated by exposure to blue or UV wavelengths (Williams and Hallock 2004). Studies of bleaching in corals indicate that decline in zooxanthellate photosynthetic capacity follows increase in daylight and precedes temperature peak (Warner et al. 2002), and that UVR and PAR exacerbate supraoptimal temperature effects (Lesser and Farrell 2004). Although MAAs are photoprotective, the energetic cost of MAA production may inhibit growth and recovery from stress (Hoogenboom et al. 2006**)**, and high solar radiation may depress MAA production (Lesser and Farrell 2004**).**

Based on modeled entire water column photobleaching in lakes, photobleaching can cause 0.6 to 1.4% decrease in CDOM light absorption over the timescale of tidal flushing (12 hours) (Reche et al. 2000). Offshore reefs and developed shoreline-associated reefs that do not receive consistent, tidally flushed pulses of CDOM are particularly susceptible to increased UV transparency due to photobleaching of CDOM.

In conclusion, UV irradiance may contribute to photooxidative stress and reef decline in the Florida Keys. Management of shorelines to protect sources of photo-protective CDOM such as mangroves, seagrasses, and wetlands may reduce susceptibility to bleaching in corals.

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Second-Generation Landscape Mosaics of Coral Reefs

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Abstract. Efficient survey methodologies that provide comprehensive assessment of reef condition are fundamental to coral reef monitoring. Current state-of-the-art techniques in coral reef assessment rely on highly trained scientific divers to measure indices of reef health (e.g., substrate cover, species richness, coral size, coral mortality). First-generation video mosaics were an innovative survey technology that provided large-scale (up to 400 m^2), spatially accurate, high-resolution images of the reef benthos without extensive survey times or a need for scientific divers. Despite these advances, first-generation mosaic products were insufficient for species-level identification of many benthic taxa, thereby limiting the monitoring potential of the technique. A second-generation mosaic survey technology has been developed, which integrates high-resolution still-image acquisition with high-definition video surveys of the reef benthos. These second-generation products have sub-millimeter benthic resolution allowing for species identification of coral colonies (as small as 3 cm), identification of macroalgal genera, and increased information on coral colony health and small scale competitive interactions. This combined survey technology allows users to collect imagery on both a landscape and colony level over 100's of square meters in under an hour of in-water dive time. The resulting product has excellent archive potential and is a superior tool for tracking changes over time.

Key words: Video mosaics, coral monitoring, benthic surveys.

Introduction

Worldwide decline of coral reef communities since the 1970's has prompted the development of numerous reef monitoring techniques at multiple spatial scales. Traditional monitoring methodologies rely heavily on expert scientific divers to measure indices of reef health during field surveys. These methods often result in long dive times and a lack of permanent raw data of the state-of-the-reef at the time of the survey. To address these weaknesses, many monitoring programs now incorporate underwater photography or video into their survey design so that images can be analyzed out of the water to measure metrics such as percent cover and coral colony sizes. Standard underwater video and still imagery often reduce the dive time and expense of reef monitoring, but they provide either a small image footprint or have low resolution thereby limiting their value as complete coral community monitoring tools.

An underwater video mosaic survey technology has been developed to create large (several hundred m^2), spatially accurate, high-resolution video mosaics of reef communities that provide a landscape view of the seabed while minimizing dive-time (usually < 1 hr) (Lirman et al. 2007). Such "landscape mosaics" overcome many limitations of previous survey methods while retaining most of their strengths. Mosaics can be created without trained, expert scientific divers, long dive times, or the use of extensive permanent markers that are normally required for repeated surveys of the same locations. Their high spatial accuracy allows accurate measurements of distances and colony size directly from the mosaics themselves (Lirman et al. 2007).

Over the past four years, landscape mosaics have been used to assess coral reef status (Lirman et al. 2007), hurricane impacts (Gleason et al. 2007), bleaching events, ship groundings, and the status of submerged cultural resources (unpubl. data).

The first-generation landscape mosaics were created from video taken with either a Sony TRV 900 DV camera hand-held by a diver, or a Point Grey Research Flea camera mounted on a Remotely Operated Vehicle. These two camera systems provided spatial resolution ranging from one to four mm/pixel, which was sufficient to identify taxonomic groups, such as stony corals, sponges, gorgonians, macroalgae and sand, but was not fine enough to allow species-level identification in most cases (Lirman et al. 2007). Organisms smaller than 5 cm in diameter were typically not detectable using first-generation mosaic products (Lirman et al. 2007).

The aim of this paper is to describe a second-generation imaging system with improved benthic resolution. This second-generation system was used to survey a permanent study plot at Brooke's Reef, Florida in June 2008. This mosaic was then compared to the first-generation mosaic acquired at this same site in June 2004.

Material and Methods

The use of cameras for benthic habitat mapping has been an active area of research over the last decade. (Negahdaripour and Madjidi 2003; Gracias et al. 2003; Singh et al. 2007). In the present research, the algorithm for creating large scale video mosaics is based on the approach described by Gracias et al. (2003) and Lirman et al. (2007). The first-generation of mosaic processing consisted of four steps using underwater video images. Mosaics were produced by 1) creating an initial estimation of camera motion using feature matching of textured areas between sequential image frames (Lowe 2004; Brown and Lowe 2003), 2) predicting and matching of non-sequential frames, 3) refinement of the camera motion estimation based on using bundle adjustment, and 4) blending of the individual video frames to create a seamless single image of the survey area (Gracias et al. 2006).

This mosaic algorithm requires a high degree of overlap between sequential images to accurately estimate camera motion. In the field, high overlap between images is achieved by using a video camera with a high rate of frame capture (i.e. 30 frames per second, fps). Video cameras, however, have relatively low image resolution (< 1 Megapixel, MP). Therefore high benthic resolution images can only be acquired with these cameras if a very small field of view is maintained during the survey. This is accomplished by maintaining a small distance from the camera to the seabed (typically 40 cm or less). Although in use for some monitoring programs (e.g. Somerfield et al. 2008), this approach does not allow for the 1.5 to 2 m disparity between the benthos and camera normally needed for accurate landscape mosaic processing (Lirman et al. 2007). Current camera technology does not provide a single reasonably priced camera system that can simultaneously capture frames at a high rate with the image resolution necessary for species level identification from 2 m above the bottom.

To overcome the resolution limits of the first-generation mosaic survey technology while maintaining the high frame rate and altitude requirements of the mosaic processing algorithms, a two-camera system was adopted. This second-generation mosaic imaging system combines information from (1) a high-definition video (HDV) camera capable of full video frame rate (30 fps) and (2) a digital still camera capable of acquiring very high-resolution images (10 MP) at low capture rates (1 fps).

The second-generation mosaic imaging system was created using commercially available components, including a Nikon D200 10 MP Digital SLR camera and Ikelite underwater housing, Sony HVR-A1U HDV video camera and Amphibico housing, and a 3.5" LCD color external monitor. All components of the enhanced mosaicing system were mounted in an aluminum/stainless steel frame (Fig. 1). The enhanced acquisition hardware allows for simultaneous collection of video and photographic stills using two independent cameras mounted on the same frame.

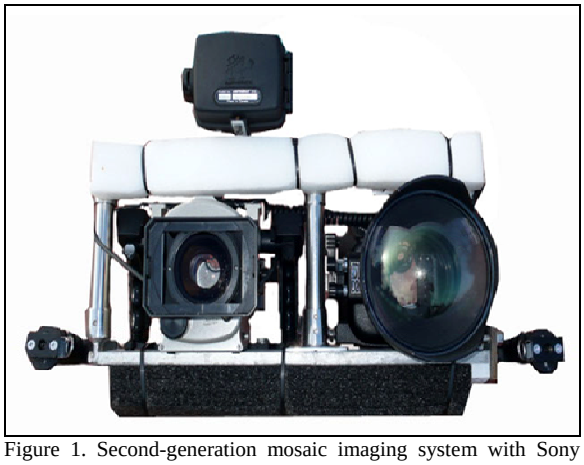

Figure 1. Second-generation mosaic imaging system with Sony HVR-A1U camera and underwater housing (left), Nikon 10 MP still and housing (right) and 3.5" LCD color video monitor (top).

In the field, the second-generation imaging system is deployed with both cameras in a down-looking position. Cameras are swum by a single diver 2 m above the reef area-of-interest in a double lawn-mower pattern as described previously (Lirman et al. 2007).

Data processing of the second-generation mosaic data is divided into two parts. First, a landscape mosaic is created using the same four steps as the previous generation of mosaic images with HDV images substituted for regular DV imagery (Lirman et al. 2007).

The second part of data processing represents a new step designed to register high resolution still images to the landscape mosaic. Automated feature matching algorithms (Lowe 2004; Brown and Lowe 2003) are used to map the still images to their geographic locations within the video mosaic. This mapping is obtained by matching individual still images to individual video frames used to create the landscape mosaic of the survey area.

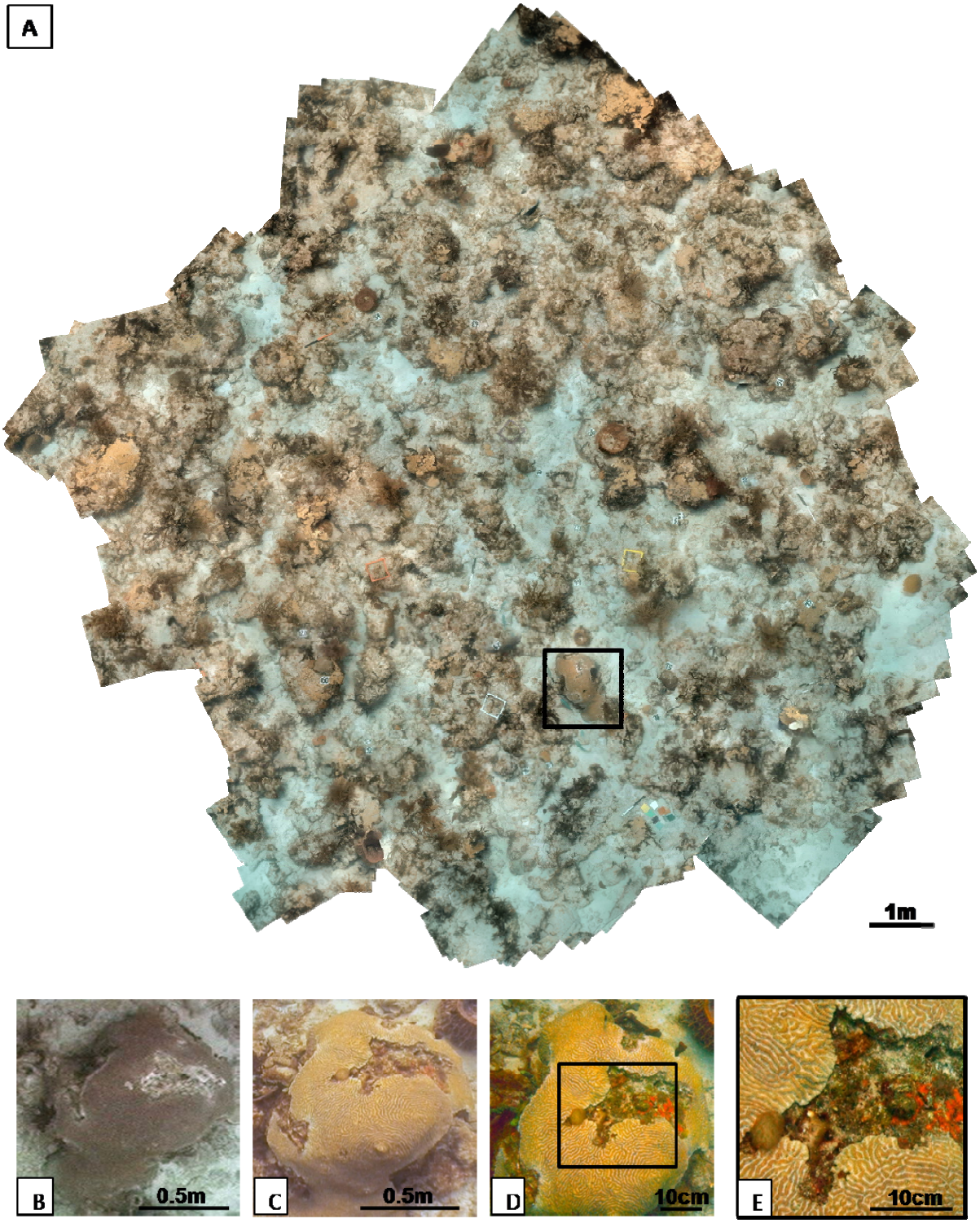

Figure 2. (A) A second-generation video mosaic from Brooke's Reef, FL taken in June 2008. Images of a *Meandrina meandrites* colony (shown in A) selected from mosaic surveys created using: (B) a Sony DV camcorder in June 2004, (C) high definition Sony camcorder from June 2008, and (D-E) 10 MP Nikon D200 still camera. All images were acquired at a distance of 2 m from the benthos. The combination of high resolution still images with video acquisition provides increased reef health information such as (D) tissue partial mortality and (E) cyanobacterial, macroalgal, and sponge interactions.

As a result of this process, each video frame used in the mosaic creation process is linked to a corresponding still image.

Display software was developed that allows users to select any point within the landscape mosaic produced by the second-generation imaging technology and retrieve the corresponding 10 MP still image and corresponding video frame of the area surrounding that point.

Results

In June 2008, a portion of Brooke's Reef, FL (25° 40.508'N, 80° 5.908'W) was mapped with the second-generation system. The landscape mosaic generated from the high-definition video of the second-generation system covered 156 m^2 (Fig. 2A). The first-generation landscape mosaic of Brooke's Reef taken in June 2004 has a spatial resolution of 3 mm/pixel (Fig. 2B), whereas the second generation version has a spatial resolution of 2 mm/pixel (Fig. 2C). The second-generation still camera images were acquired at a frame rate of 1 fps, resulting in approximately 1,800 10 MP images of the reef benthos. The 1,800 still images were then matched to key video frames. The average spatial resolution of the still images from the second-generation system was 0.4 mm/pixel, almost an order of magnitude improvement over the video of the first generation system (Fig. 2D, E).

The 2 mm/pixel spatial resolution of the second-generation landscape mosaic, produced from the high-definition video, enabled improved analysis of the mosaic. Boundaries between sponges, corals and gorgonians were clearer and large colony identification (> 25 cm) was possible for most Caribbean species (Fig. 2C).

Analysis of the 10 MP still images from the second-generation system provided the greatest taxonomic resolution. The sub-millimeter resolution of the still images allowed for species-level identification of colonies as small as 3 cm in diameter. In addition macroalgal groups were identifiable to genus with species level identification possible for macroalgae with obvious defining characteristics such as *Halimeda tuna* and *H. opuntia*. Coral colony health information such as partial mortality boundaries and evidence of bleaching and disease were all recognizable using the still images. Small-scale indicators of reef health such as cyanobacteria, macroalgal, and sponge competition were also visible using high-resolution still data (Fig 2D, E).

Discussion

The first-generation video mosaics demonstrated that mosaic-based monitoring could be accomplished without trained, expert scientific divers, long dive times, or extensive tagging of coral colonies. This technique also allowed users to assess basic reef health indices and monitor coral colonies using a large-scale image-based approach that limited the need for extensive dive-time and provided long lasting visual information on the state-of the reef at the time of the survey (Lirman et al. 2007).

Second-generation mosaics retain these monitoring strengths while providing increased benthic resolution over the entire survey area, enabling species level information for most coral colonies. This allows greater taxonomic information with respect to percent cover and diversity indices than was available from first-generation mosaics. In addition, change detection

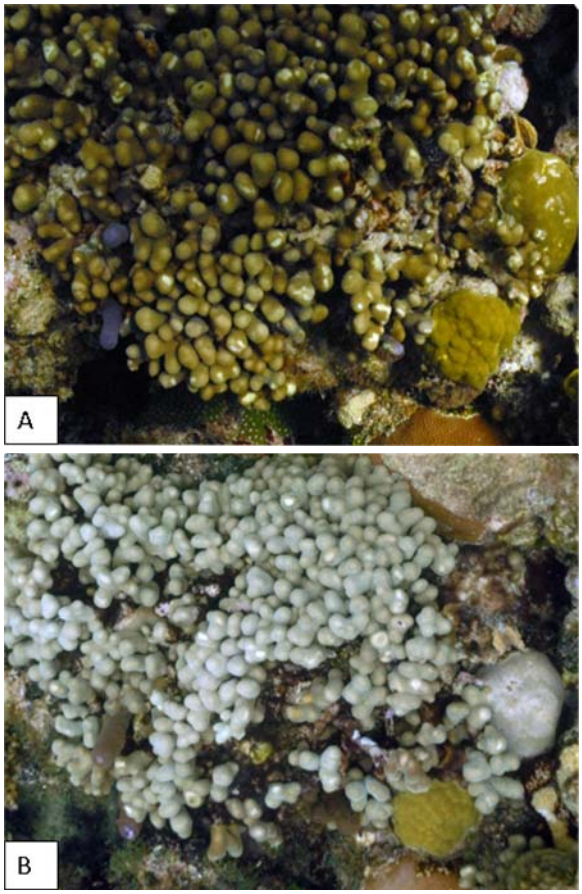

Figure 3. Example of change detection potential using second-generation imagery. High resolution still images were acquired from approximately 2 m water depth during video mosaic surveys. Images were automatically matched to their geographic locations within the survey area for change detection analyses. Between February, 2008 (A) and September, 2008 (B) the *Porites porites* (center) and *Stephanocoenia intersepta* (right) colonies shown above have undergone considerable paling. Evidence of partial mortality and an increase of cyanobacteria within the *P. porites* colony are also identifiable by comparing the survey images. Small indicators of coral health such as fish bite predation are also clearly present on both the *P. porites* and the *S. intersepta* in February 2008 and can be followed through time to monitor long-term impacts (A).

analysis using second-generation mosaic products provides information on conspicuous indices of reef health (such as bleaching and tissue loss) while also allowing users to monitor fine scale stressors such as macroalgal and cyanobacterial interactions and predation events (Fig. 3). This represents a significant monitoring improvement over first-generation products that could only discern dramatic indicators of colony health such as bleaching, and significant tissue loss (Gleason et al. 2007).

Although many monitoring programs take pictures of the reef for analysis and documentation purposes, the extensive cataloging and database management required in the laboratory often limits the amount of photographic data acquired during a survey. The automated matching of high resolution still images to their geographic location within a second-generation mosaic removes the need for user-intensive laboratory cataloging and allows the archiving of several thousand high resolution images of a reef area with minimal user input.

Image-based techniques for reef monitoring have an excellent archive potential that is vastly superior to written notes by divers. The second-generation mosaic survey technology described here provides both landscape level and colony level (as small as 3 cm) permanent records of the state-of-the-reef at the time of the survey without increasing the dive-time of first-generation mosaic products. The simultaneous capture of both of these scales of information allows users to monitor large-scale changes in reef communities such as hurricane or ship grounding damage, as well as small scale interactions such as sponge and macroalgal competition using the same survey design and the same raw data set.

Conclusions

The incorporation of high-resolution still imagery into the second-generation imaging equipment and mosaic processing resolved the species identification limitations of the first-generation system.

The ability to capture high resolution images over 100's of square meters and georeference these images within video mosaics without user input is a major advantage of the landscape mosaic technology. The combination of large-scale image maps and high-resolution benthic information provides a unique tool that maintains the strengths of most traditional monitoring methods, such as the ability to extract indices of reef health (percent cover, coral colony sizes, and diversity indices), while also providing a rapid method to document and assess changes at both large and small scales in coral communities without extensive tagging. This combined with the extensive archive potential inherent to this technology provides a significant advancement in coral reef community monitoring technology.

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The Light and Motion Sensor Program: Low cost coral reef monitoring

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Abstract
Effective coral reef management requires early identifications of potential sources of contaminants before significant degradation of the reefs occur, allowing stakeholders, politicians, businesses and concerned citizens to have sufficient information with which to take appropriate, timely remedial action. The Rainbow Sensor program can provide a reliable, continuous, low-cost measurement of the organic content of seawater, needed by managers in economically important marine protected areas to monitor basic water quality information near significant human development. Increases in organic content can come from the addition of nutrients and hence algae growth or dissolved and particulate organic contaminants from land. The Rainbow Sensor uses spectral optical attenuation of light through the water column to detect the presence of shore based, human generated contamination. The first implementation of the moored Rainbow Sensor, the Light and Motion Sensor Program (LMSP) on Bonaire has, in its first year, shown that it can detect organic matter in the water column. The low cost (< US $1500) of each Rainbow Sensor mooring brings the capability of continuous, long-term measurement of the organic content of seawater to many agencies that do not have the budget for more expensive traditional monitoring systems.

Key Words: organic matter, contaminants, monitoring, light attenuation, water quality

Introduction

Coral reefs are vulnerable to a range of environmental effects, from the local to global scales. The local scale includes several types of effects. Removal of fish or shifts in population structure due to invasive species (top-down effects) can result in loss of grazing pressure on reef algae (Littler et al. 2006). Reefs can also be affected by mechanical damage. Mechanical damage can come from either human activities that include construction, boat anchors or diving activity or from natural processes such as wave damage during hurricanes. Bottom up effects come from modification of the base of the food web such as increased algal growth due to nutrient input, contaminants, or other processes that will affect the lower trophic levels (e.g., Lapointe 1997). Nutrient and contaminant inputs are usually associated with anthropogenic sources that increase with increasing population pressure that has resulted both from modernization and population migration to coastal regions. Regional processes often include fluctuations in regional circulation, eddies, coastal upwelling, storm systems, etc. Global processes include global climate change (IPCC 2001), ocean acidification (Caldeira et al. 2007; Feely et al. 2004), and global climatic oscillations. Climate variaibility has been suggested as a possible mechanism for rapidly altering the state of the ecosystem (Scheffer et al. 2008). Local processes, usually the most manageable processes, are often associated with human activities that include coastal modification, watershed and land use changes, sewage discharge, etc.

Maintaining as healthy a reef system as possible by local intervention may offset some of the effects of changing global and regional phenomena. Providing marine protected area managers with indications of increasing contamination levels that could degrade a reef enables them to seek effective solutions, ideally, before significant degradation occurs or becomes noticeable. Effective coral reef management, then, requires that contaminants are detected and identified before significant reef degradation can occur, so that the stakeholders can understand the presence and threat to a reef system and take preventive action as rapidly as possible.

The Light and Motion Sensor Program (LMSP) was developed to provide an inexpensive monitoring tool that will detect the presence and/or effects of contaminants in the water column. Anthropogenic inputs from runoff and sewage discharge into the coastal ocean (whether intentional or inadvertent) contribute inorganic nutrients contributing to algal growth, and organic matter that may alter the trophic structure of a reef (e.g., Lapointe 1997, 1999; Littler et al. 2006). Both organic matter and planktonic algae absorb light more strongly in the blue region of the spectrum than seawater (Kirk 1994; Fig. 1). Runoff and sewage discharges are characterized by increased colored dissolved organic matter (CDOM), suspended particulate matter and nutrient inputs (e.g., Petrenko et al. 1997; Twardowski and Donaghay 2001; Wu et al. 1994). Thus, it should be possible to create an index of the organic matter content of the water column, the organic index.

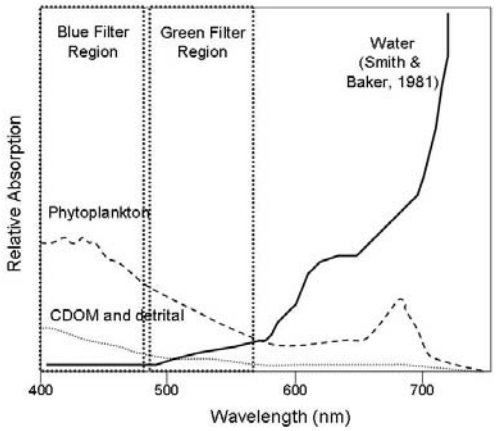

Figure 1: Sketch of Rainbow Sensor absorption spectrum. X-axis: light spectrum; Y-axis: Relative absorption of light in the water.

In clear oligotrophic water, attenuation in the green portion of the spectrum is typically higher than attenuation in the blue portion of the spectrum (Smith and Baker 1981). In the blue range, K_{Ed} increases due to increased absorption by organic matter while the green K_{Ed} is largely unaffected. Therefore, the Organic Index (OI) is correlated with: chlorophyll concentration (phytoplankton biomass), CDOM, and particulate organic matter.

This paper describes this approach and provides examples from the first year of implementation that indicate the utility of this monitoring system.

Material and Methods

The Light and Motion Sensor Program on Bonaire uses the Rainbow Sensor array developed at the University of Southern California to measure K_{Ed} for blue, green and broadband white light from sensors at three depths. The program uses Hobo Pendant temperature/light sensors (Onset Computer) with transparent colored filters inserted in two of the sensors. The three sensors are placed in an array at a specific depth below the surface (Fig. 2). On the LMSP moorings sensors are placed at 5 meter, 12 meter and 20 meter depths, for a total of nine sensors per mooring. The sensors are placed face up so that they are measuring the downwelling irradiance, E_d, at each depth for each spectral range.

Figure 2: LMSP Rainbow Sensor Array on a mooring line in the Bonaire National Marine Park.

The filters currently used by the LMSP program are blue and green filters with defined optical characteristics manufactured by Lee Filters, Inc. (http://leefilters.com). Figure 3 shows the transmission characteristics of the blue light filter. It transmits about 35-55% of the light between 400 and 450 nm. This portion of the spectrum is where CDOM and chlorophyll strongly absorb. The filter also effectively transmits light at 700 nm. However, water rapidly attenuates red light (> 600nm) in the upper few meters of the water column. At 700 nm, the seawater attenuation coefficient is 0.65 m^{-1} in the clearest seawater (Smith and Baker 1981), attenuating downwelling irradiance to 4% of its surface value at 5 meters depth. While there may be slight contamination of the blue light with red light using this filter at 5m, by 12 meters depth the downwelling irradiance at 700nm is reduced to ~0.04% of surface light.

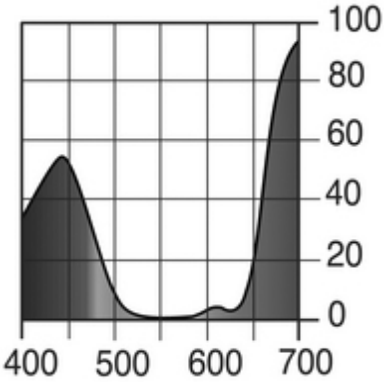

Figure 3: Blue filter transmission spectrum. The x-axis is the wavelength in nanometers and the y-axis is percent transmission. The spectrum is for the Lee Filter #798, Chrysalis Pink filter (http://www.leefilters.com/).

Figure 4 shows the green filter spectrum. The filter permits very little light in the blue range (< 470nm) but allows the entire green range (470-570nm) to pass. In this range organic matter affects light attenuation much less than at shorter wavelengths.

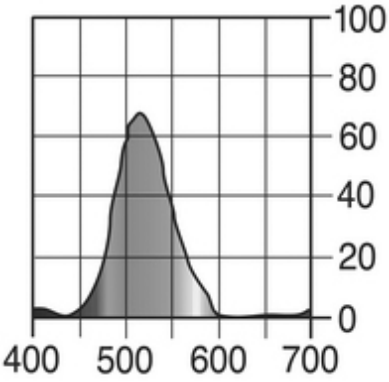

Figure 4: Filter transmission spectrum for Lee Filter #124, Dark Green (http://www.leefilters.com/). The x-axis and y-axis are the same as in Figure 3.

The sensors are programmed to measure temperature and light continuously at one minute intervals. Volunteer divers use an underwater optical reader to download data and re-launch the sensors on each mooring weekly. This data is transmitted to the University of Southern California in Los Angeles, California. Since the sensors are not self cleaning, the volunteers clean the sensors after downloading the data.

For each depth pair the diffuse downwelling attenuation coefficient [$K_{Ed}(\lambda)$] is calculated from the downwelling irradiance for each color region using equation 1.

$$K_{Ed}(\lambda) = -\frac{\ln\left(E_d(\lambda, z_2) \big/ E_d(\lambda, z_1)\right)}{z_2 - z_1} \quad (1)$$

where:

$Ed(\lambda,z)$ = downwelling (downward propagating) irradiance at wavelength λ at depth z

$K_{Ed}(\lambda)$ = diffuse downwelling light attenuation coefficient.

For each mooring nine K_{Ed}'s are calculated, blue, green and white sensor pairs for the depth intervals of 5-12m, 12-20m, and 5-20m.

The LMSP on Bonaire currently (September 2008) employs twelve moorings, each with nine sensors, distributed along the western leeward coast (Fig. 5). Two more moorings are expected before November 2008.

Figure 5: Locations of LMSP moorings on Bonaire along the central leeward coast.

Results

The Light and Motion Sensor Program on Bonaire has, in its first year, yielded results that show several processes that affect both the temperature and water quality on the reef. One of the moorings was located at a dive site called "Front Porch", indicated by #1 in Figure 5. During January 2008, the water column cooled by nearly 1.5°C accompanied by a doubling of the optical index (OI), calculated as the difference between K_{Ed} (blue) and K_{Ed} (green) (Fig. 6).

The temperature cooling suggested that this was likely an oceanographic process that was causing the change in the optical attenuation. MODIS-Aqua remotely sensed sea surface temperature (SST) and chlorophyll imagery (University of South Florida, Center for Remote Sensing) showed cool, chlorophyll-containing surface water extending northward from the Venezuelan coast, apparently due to coastal upwelling. Thus the cooling and increasing

OI on Bonaire advected from the Venezuelan coast to Bonaire, increasing the organic index resulted due to increased chlorophyll associated with the upwelled water.

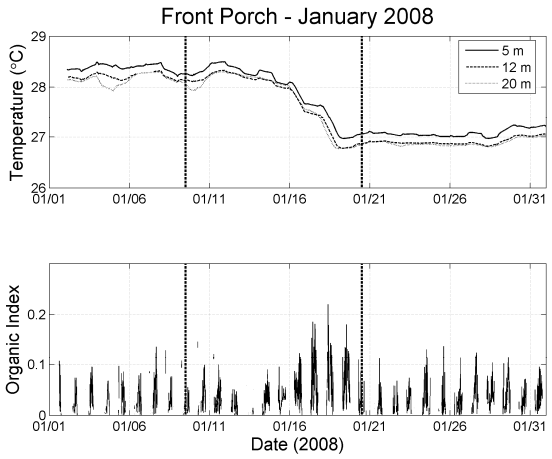

Figure 6: Time-series of temperature (top graph) and organic index (bottom graph) ($K_{\mathbf{Ed}}$ (blue, 12-20) –$K_{\mathbf{Ed}}$ (green,12-20)) for Site #1 (see Figure 2) in January 2008.

In the second example from the LMSP data set the seasonal variation from October 2007 through June 2008 is examined. During this period, K_{Ed}(blue) was often less than K_{Ed}(green), giving a negative organic index.. We therefore show only K_{Ed}(blue), which by itself is as an index of organic matter because of the strong blue absorption by phytoplankton and dissolved organic matter in the blue portion of the spectrum (Fig. 1). The time series showed a general increase in the K_{Ed}(blue) along the entire developed coast of Bonaire between November 2007 and April 2008 (Fig. 7).

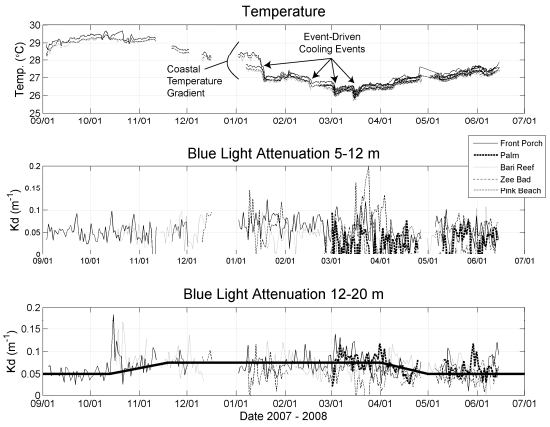

Figure 7: Example of possible local processes along the Bonaire coast. This includes data from 5 moorings.

This period coincides with seasonal cooling and is also concurrent with the highest tourist activity. The general pattern is consistent across several sites, suggesting that the increase in K_{Ed}(blue) is not due to localized contamination at any single site, and may likely result from increased mixing and primary productivity during the winter months. The K_{Ed}(blue) values in this time series are consistent with observations off the northwest coast of South America away from the influence of the Amazon River plume (Del Vecchio and Subramaniam 2004).

The observed seasonal cooling is not a continuous process, but is characterized by several step-wise cooling events of varying magnitude. The temperature data has been particularly useful in understanding the local and regional mechanisms that affect the reef.

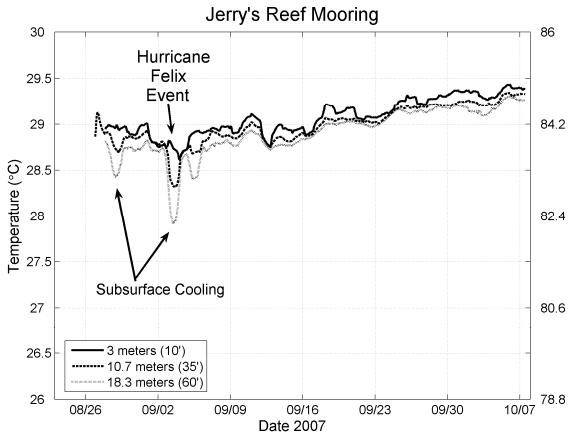

Figure 8: Vertical temperature structure during a near pass by hurricane Felix on September 2, 2007. The mooring site is Jerry's Reef near island of Klein Bonaire.

Figure 8 shows the effects of the passage of a hurricane event on the subsurface temperature. The temperature time series indicates that the cooling occurred from below rather than mixing downward from the surface. Cooling at 20 m preceded slightly the cooling at shallower depths. This contrasts with the cooling shown in Figure 7 where temperatures at all depths converged and the cooling event in Figure 6 where temperatures fell proportionally. By September 9, the effects of the event had passed and seasonal warming continued.

Discussion

After its first year of implementation, the Rainbow Sensor array has demonstrated that it can detect events in both temperature and in the diffuse attenuation coefficient in the blue portion of the spectrum. Two patterns in blue absorption were observed. One was a response to regional coastal upwelling off of Venezuela that could be seen in the remote sensing imagery to advect cool, chlorophyll enriched water toward Bonaire. The second pattern was a seasonal pattern where higher values of

K_{Ed}(blue) were observed during winter months than during spring/early summer. We are uncertain of the cause of the winter increase of attenuation, but nevertheless, the moorings were able to detect the pattern.

Improvements to the Rainbow Sensor array continue. The filter that is being used for the blue measurements also transmits light in the red region of the spectrum (Fig. 4). Although the downwelling irradiance at 700 nm is reduced to 4% of surface light at 5 meters, it may still cause a small amount of contamination in the total light and bias the K_{Ed}(blue) values higher because of the high values of red attenuation by water itself. We are looking for a suitable filter that has adequate transmission in the blue portion of the spectrum and minimal transmission in the green and red parts of the visible spectrum.

The low cost of the moorings (< US $1500) provides the possibility of continuous, long-tern measurement of the organic content of seawater to agencies that do not have the budget for more expensive traditional instrument systems. Manufacturers such as WETLabs and Satlantic produce *in situ* nutrient sensors that cost US $15-20,000 each. Similarly, CDOM and chlorophyll fluorometers cost about US $3500 each and require additional battery packs and loggers for moored applications. Although these more expensive instruments provide specific, accurate measurements, the costs of implementing a mooring with these sensors may rapidly become prohibitive.

This approach is applicable to coastal reef systems near human development where the effect of human generated pollutants could affect the health and economic viability of the reef. The approach is best suited for coral reefs at greater than 5-10 meters depth and where volunteers and/or low cost individuals that are only recreational SCUBA qualified are available and allowed to service the sensors.

We are continuously improving the procedure for the Rainbow Sensor and for analysis of the time series of temperature and K_{Ed}. We welcome the opportunity to collaborate with other groups interested in implementing this approach.

We conclude that the "Rainbow Sensor" concept appears to provide a reasonable measure of K_{Ed} in the blue portion of the spectrum. Because most of the variability between 400 and 490 nm is associated with organic matter in various forms including CDOM, chlorophyll and particulate organic material, variability of K_{ed} in this region of the spectrum should represent changes in the organic content of the water column. We believe that the Rainbow Sensor array is an effective, inexpensive approach to monitoring changes from contamination that might affect the reef ecosystem. Because it records its data at a reasonably high frequency, it provides the ability to resolve processes over a range of time scales range from minutes to years, depending on the lifetime of the moorings.

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COREweb, a web-based information management solution for experimental data from the field of coral reef ecology

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Abstract. In the profit-oriented business and industry sector, information management (IM) solutions are well established. But in coral reef ecology, the IM aspects of experimental design, data acquisition and evaluation, documentation and publication follow the individual workers' experience rather than established standards. Although these tasks are largely automatable, no useable guidance tool has been established so far that would lead the worker through the experimental life cycle and enforce validation, documentation, completeness, correctness and consistency of data. Often, this causes loss of information and diminishes data quality as well as compatibility. We developed COREweb, a dynamic web application to automate the scientific work process in consistency with established scientific work practices. The underlying data model is capable of managing any kind of manipulative or monitoring ecological experiment. The user operates COREweb through an intuitive Graphical User Interface and focuses on the scientific experimentation and validation process. Data can be shared among team members. COREweb is scalable from a single-user, single-desktop environment up to a distributed network, connecting teams worldwide. As COREweb is easily adapted to many different scientific experiments, it can serve as a prototype model for useable scientific IM tools that make valuable data better available to decision makers.

Keywords: Scientific work flow, process model, data model, information management

Introduction

In life sciences, researchers proximately want to publish as fast and high-ranked as possible with the least effort of time, financial and personal resources. Therefore, any means of facilitating and improving the scientific work flow should be in their best interest. Information management (IM) is the collection and management, *i.e.* the organisation and control of the structure, processing and delivery of information. Information in research projects consists of the experimental data itself, the metadata, instructions on methodology, best practices, abided protocols and standards as well as any uncategorized information which ultimately helps a research team to gain knowledge. A rising number of collaborators, close and remote, requires means of online collaboration and tools to share data and knowledge. The following tools and standards exist for this purpose.

Metadata and data standards The ecological markup language (EML) is an extensible markup language (XML) extension that serves to document and handle ecological metadata (http://knb.ecoinformatics.org/software/eml/). Metadata are needed for the identification and documentation of the multitude of heterogeneous ecological and environmental data sets. However, proper documentation via metadata depends on the individual worker's motivation and is therefore not very prevalent.

In some software packages, such as ECOBAS (Benz et al. 2001), data completeness, correctness and consistency is automatically enforced by the package's functionality.

Experimental design and data modelling Calvin Dytham presented a simple-to-use decision tree to guide the scientist from the formulation of their research questions towards the choice of an appropriate statistical test (Dytham 2003).

The software *Touchstone* allows the user to refine the experimental design in a "what if" style until a statistically satisfying setup has been found (Mackay et al. 2007).

Pratt (1995) designed a relational database schema which can store the data of empirical studies. The schema bases on a model of an empirical study of natural processes.

Work flows and method provenance Kepler automates work flows, i.e. the processing steps from raw to analyzed data (Bowers et al. 2006), and features a model for user-oriented data provenance in pipelined scientific workflows. Provenance information may also be used by scientists to reproduce results from earlier runs, to explain unexpected results, and to prepare results for publication. Bowers et al. (2006) developed a simple provenance model that is capable of supporting a wide range of applications even for complex models of computation, such as process networks.

The *Karma* provenance framework provides a means to collect workflow, process, and data provenance from data-driven scientific workflows (Simmhan et al. 2006) in order to retain the knowledge that was put into the experimental planning and refinement.

Data lineage Bose and Frew (2005) review the data quality benefits of lineage, i.e. the documentation of data origin and processing history. Lineage helps to understand data processing steps (Woodruff et al. 1997), enhances interpretation, prevents misinterpretation of data and communicates data suitability, reliability, accuracy, currency and redundancy (Eagan et al. 1993). Lineage also facilitates the use of historical data (Clarke and Clark 1995).

Process models In software design, several procedures exist which structure and guide the process of coding new programs. The V-model (Boehm 1979) is one approach that parallels ecological experiments.

In the V-model, the initial steps of conceptual planning preceding the actual writing of source code are designed with regard to the requirements of the later steps of testing and evaluating the software. The writing of source code begins only once its desired functionality is defined and understood.

Since the V-model's "think-first" approach helps eliminating ambiguity and identifying non-trivial requirements, the time spent with planning ahead pays off at the stage of generating the source code.

Similarly, the researcher has to design manipulation and observation routines with regard to the statistical power of the generated data in order to avoid generating data with a low statistical power and, therefore, losing valuable time and financial resources.

Aim of this study This study aims to incorporate these established standards and best practices into a process model of an ecological experiment by abstracting the ecological work process and its related artefacts into formal languages. This is achieved through the graphical user interface (GUI) structure and database schema of a database-driven web application, which provides modular tools that guide and assist the user through the experiment's life cycle.

Materials and Methods

Via model-driven development (MDD), COREweb was generated to a large extent from the formal language artefacts using Grails, a MDD framework (Rocher et al. 2006). COREweb is a model-view-controller (MVC) web application, which provides a separation of the database structure (model), the GUI (view) and the functionality (controller).

The COREweb prototype currently supports the process up to the generation of the data structure. It is possible to setup and thoroughly document the experiment, enter the raw data with input validation at a user-defined level and export the validated, well documented data and metadata for processing and statistical analysis.

COREweb is deployed as a Java applet, connected to a postgres database, runs on a web server and can be accessed in any browser via the inter- or intranet. The database and the java applet can run on one or on different machines. Source code, technical documentation and a walkthrough using an example experiment are available at http://palmuc.de/core/coreweb/ .

Results

Abstracting the experiment into a database schema

Methods consist of several manipulative or observational steps. Samples are the individual experimental units, which are manipulated and observed. Observation results in the measurement of their properties.

A sample experiment is shown in Fig. 1; two methodical steps result in a total of five different measurements. The sum of measurements and the number of samples define the data structure, paralleling the columns (measurements) and rows (samples) of a spreadsheet.

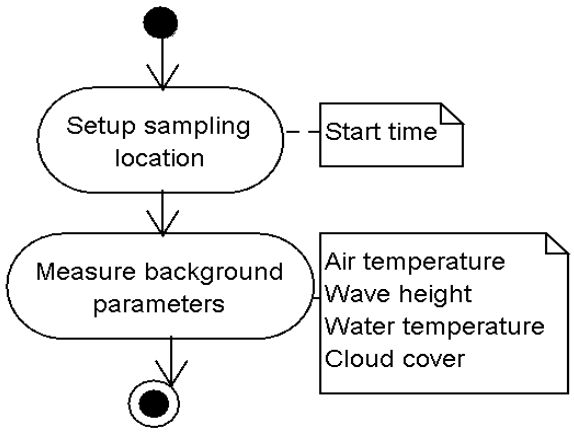

Figure 1: A sample experiment with two methodical steps (round boxes) and five measured parameters (rectangular boxes).

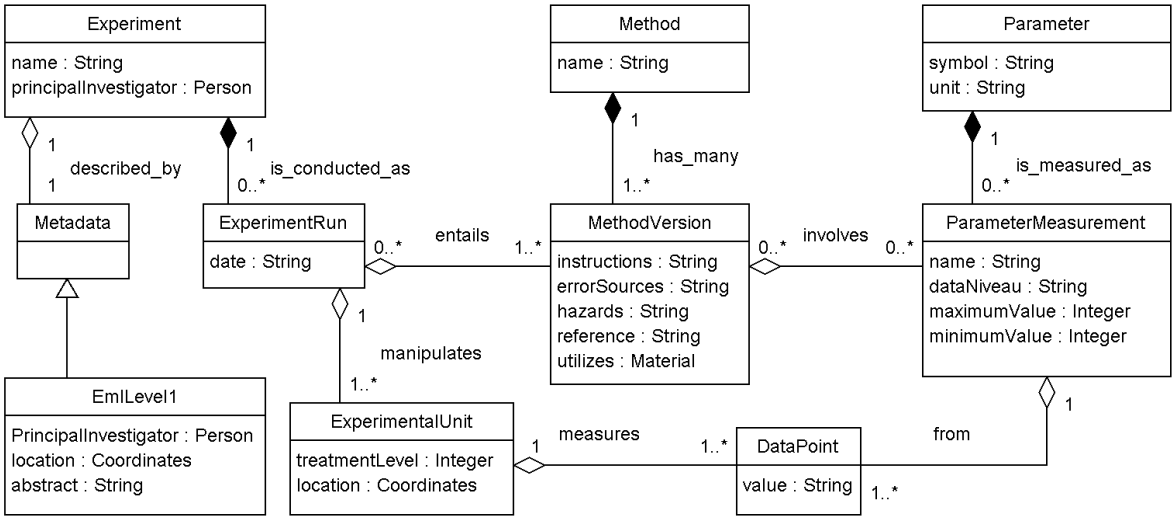

Figure 2: Simplified data model of an abstracted scientific experiment, shown as a UML class diagram. Each class, represented by a box, corresponds to a data table in the database backend. Each relation between classes, represented by a connecting line, corresponds to a foreign key in the database backend.

A database schema was designed that can store information on metadata, documentation, methodology, information on experiments, their individual realizations (runs), the samples and the measured parameters by only adding entries to the existing tables (Fig. 2).

In contrast to conventional spreadsheets, the data points are linked to their methodical origins (MethodVersion) and metadata documentation, providing data lineage, method provenance and semantics via metadata.

The database backend itself is hidden from the user, who interacts with the application through an intuitive GUI front end (Fig. 3), which unobtrusively offers best-practice functionalities at the appropriate process stages of the experiment and implements standards, e.g. the EML metadata format. MDD allowed a large portion of code to be generated from the database schema.

Process model of an ecological experiment Paralleling the V-model, a proposed work flow of an ecological experiment, based on manipulation and observation, was modelled in the unified modelling language (UML, information and tutorials at **http://www.uml.org/** , Fig. 4). It features iterative testing using automatically generated dummy data, automation of the data processing and evaluation steps and refinement of the experimental design. It focuses on the statistical power of the generated data.

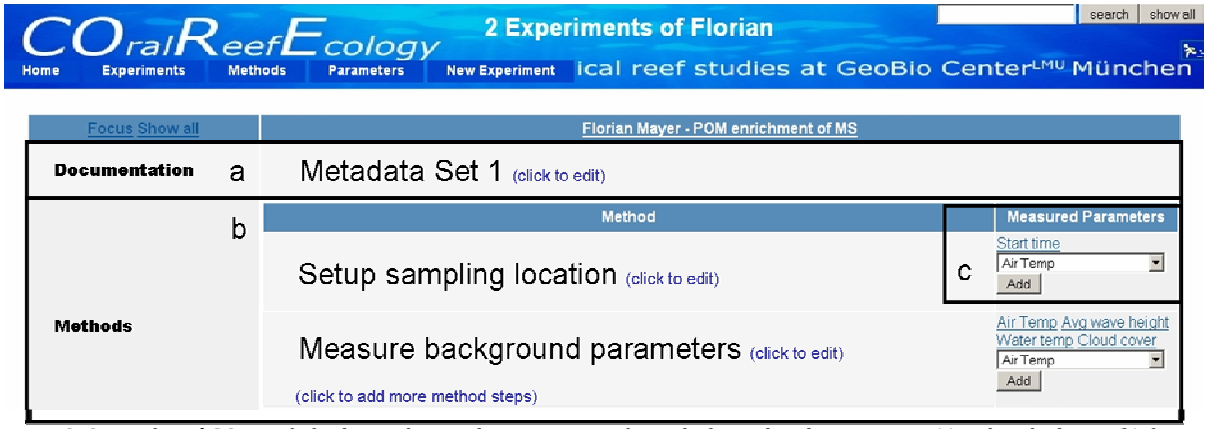

Figure 3: Screenshot of COREweb displaying the sample experiment with attached metadata documentation (a) and method steps (b) during which several parameters (c) are measured.

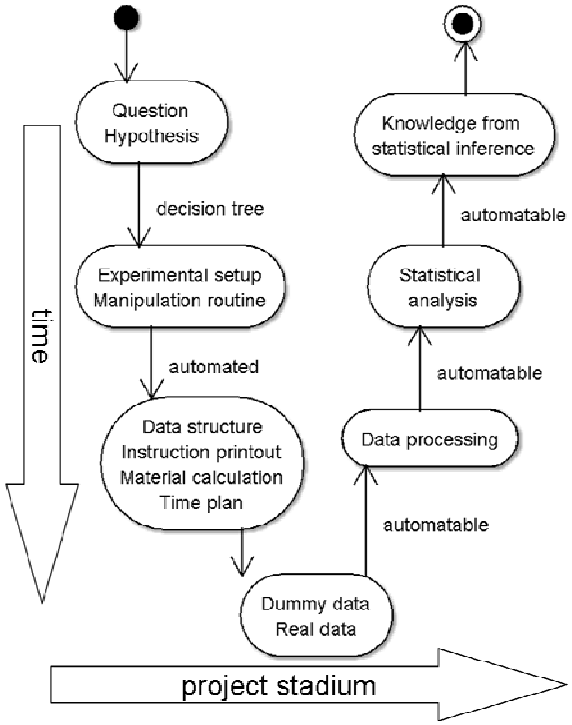

Figure 4: Process model of an experiment. The process can be dry-run and refined using dummy data. Real data should be generated after the "dry-run" validation of the process.

The proposed work flow consists of:

(1) The initial description of the planned experiment and formulation of the questions and hypotheses. Input masks channel this information into valid EML metadata; reporting functions produce an initial project outline from the entered information.

(2) Choosing the appropriate statistics to test the hypotheses following a decision tree adapted from Dytham (2003), leading to a possible refinement of the hypothesis and, ultimately, to the statistical constraints on the experimental setup.

(3) Choosing and documenting the experimental setup, the manipulation and sampling methods. Export functions provide methodical instructions, material requirement lists, a Gantt-chart style time plan, the sampling scheme, lineage documentation and the resulting data structure in form of a data table. Figure 5 shows the data table resulting from the sample experiment shown in Fig. 1. At this stage, the user can map the statistical constraints (e.g., one grouping variable, one affector and one responder variable *sensu* Pratt (1995)) onto the respective parameters.

(4) Validation of the planned experiment using automatically generated dummy data in form of a separate "experimental run." Implementation of an export into comma separated value (CSV) spreadsheets is pending.

(5) Transformation of raw dummy or real data into processed, analyzable data. This step is potentially automatable via workflows (e.g. using *Kepler*). Bulk import of existing data, such as sensor data, will be implemented in this step.

(6) Statistical analysis of the data for inference and evaluation of the hypotheses. Pipelining the data and desired statistics as input arguments; the analysis in the software package R can be automated as well as the export to CSV spreadsheets. As the least common denominator file format, CSV can be read by most analysis packages.

(7) Interpretation of the statistical output and transformation of the analysis into knowledge.

Up to this stage, dry-testing the process with dummy data entails continued documentation and refinement of the experiment. As soon as the experiment is validated and the statistical power of the experimental setup is satisfactory, the process steps 1 - 7 can be used to conduct the real experiment. The EML metadata will be made exportable to a readable table format using style sheets as well as to established metadata databases.

Service-oriented architecture (SOA) The single work steps are implemented as optional services, so the user can choose between guidance and self-determination. COREweb functions as a framework with plug-ins of existing technology; the least common denominator data structure (see Fig. 2) makes data and information compatible between the modules.

	Setup sampling location - Start time	Measure bg parameters - Air temp	Measure bg parameters - Wave ht	Measure bg parameters - Water temp	Measure bg parameters - Cloud cover
Sample 1					
Sample 2					
Sample 3					
Control 1					

Figure 5: The resulting data table from the sample experiment (see Figs. 1 and 3). The column headers are tagged with the respective method and parameter names.

Discussion

Advantages of software-conveyed guidance The database schema of COREweb is able to store raw data from ecological experiments. In combination with appropriate metadata documentation, results compatibility and transparency of data sets.

Data completeness, correctness and consistency can be easily enhanced by the GUI's input validation. For example, by simply filling in the metadata form fields, the user creates valid EML metadata without having to know EML. Similarly, the user can determine the data type of the measured parameters. From these constraints, the software can validate input, e.g. warn if characters are entered in a date field.

Automation and simulation via dummy data encourages the user to simulate the experiment in advance and to subsequently refine the planned process. This may save time and financial resources, if e.g. the number of samples can be optimized.

Early documentation creates a quick overview of the planned methodology. Incremental refinement of both the documentation and the methodology provenance *sensu* Bose and Frew (2005) provide advantages for the team in terms of consistent methodology and knowledge exchange. It also leads to raised data compatibility and quality.

Assigning the statistical relevant terms (e.g., grouping variable, affector, responder) onto the data structure helps to document the experiment's semantics.

Following a user interface's work flow implicitly and unobtrusively guides the user along the implemented standards and best practices.

Challenges for a computerized IM solution Mueller (1994) after early studies with computerized heuristic tools for engineers found that "it is not in the human nature to work rationally dominated". Until now, no IM solution successfully integrates standards and best practices into the scientific work process. This may indicate that on the one hand, known benefits of adhering to those standards and best practices do not feed back quickly or tangible enough into a speed-up or improvement of the user's own work process. On the other hand, Jagadish et al. (2007) emphasized the lack of usability of recent IM software. For non-scientific use however, intuitive and very well useable web applications exist, such as social networking platforms, which collect user-generated data and provide connectivity by addressing the users' play instinct and acquisitiveness. COREweb tries to overcome the user's initial reluctance with tangible rewards, e.g. documentation spin-offs like material calculation lists, time plans and field instructions that can be set up quickly and refined subsequently as well as with a good usability.

Mueller (1994) also found that his test subjects did not use software solutions voluntarily. Our SOA approach leaves most guiding functionalities optional; therefore, the user retains maximum autonomy.

Although the GUI of COREweb was purposefully kept simple in order to focus on development of the key functionalities, the ongoing implementation as an MVC web applet allows a refinement of the GUI towards a smooth and increasingly intuitive interface.

Summary

The software prototype COREweb implements core functionalities of the scientific work flow, implicitly integrating established standards and best practices.

Via automation of the data processing and analysis steps, COREweb facilitates a test-first approach using dummy data. Documentation rewards the user through the feedback loop of useful printout artefacts, such as material calculation lists, time plans or work instruction sheets.

Further development of COREweb will increase usability and smoothness of the work flow as well as it will implement more functionality.

Acknowledgements

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Monitoring coral reefs in optically-deep waters

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Abstract. Although remote sensing technology is a useful tool for monitoring shallow (<20 m depth) coral reefs present in clear oligothrophic waters, the large-scale assessment of reefs present in optically-deep waters requires other approaches such as the use of *in situ* platforms for high-resolution optical and acoustic imaging. Optically-deep waters are those where the upwelling radiance received by the airborne or satellite sensor originates from the water column without any bottom signal contribution. The Seabed autonomous underwater vehicle (AUV) is an ideal platform for mapping and characterizing mesophotic reefs, those present between 30 to about 100 m depth, and for shallow reefs in turbid areas. This AUV was utilized to acquire high-resolution digital images to characterize the geomorphology and dominant benthic components present along two long photo-transects in southwestern Puerto Rico. These quantitative AUV surveys also provide a baseline for future evaluation of change in the deeper coral reef zones, which for most areas of the world, remain largely unknown.

Key words: Mesophotic reefs, Seabed AUV, Puerto Rico, optically-deep waters

Introduction

Coral reefs, one of the most productive and diverse of all ecosystems, are increasingly at risk due to anthropogenic effects and global climate change. Although the use of remote sensing from aircrafts and satellites has been successfully used for mapping and monitoring coral reefs, there are many limitations in the use of this technology. For instance, the spectral and spatial resolution of existing sensors, the confounding effects of variable water optical properties and bathymetry are significant obstacles for the implementation of this technology.

Other approaches such as *in situ* platforms and sensors are required for benthic habitat mapping in turbid or optically-deep waters (i.e. where the signal received by the airborne or satellite sensor originates from the water column without any bottom signal contribution). This is also the case for mesophotic coral reefs (30-100 m) present in clear but deep waters, where the exponential attenuation by the water column precludes the use of optical remote sensing. For quantitative assessments of these deeper reefs we have used the Seabed autonomous underwater vehicle (AUV), which was designed for high-resolution optical and acoustic imaging. As a leading instrument in this field, the Seabed AUV has been successfully deployed for characterizing deep coral reef zones in the US Virgin Islands (USVI) and Puerto Rico (Armstrong et al. 2002; Singh et al. 2004; Armstrong et al. 2006; Armstrong 2007). The digital photo transects obtained by the Seabed AUV in these studies provided quantitative data on living coral, sponge, gorgonian, and macroalgal cover as well as coral species richness and diversity. Previously undescribed, well-developed coral reefs with 43% mean living coral cover were found at depths of 40-47 m south of St. Thomas, USVI (Armstrong et al. 2006).

Five digital phototransects of the upper insular slope at La Parguera and Guánica, southwestern Puerto Rico, were obtained in 2004 by the Seabed AUV. Results from the first two AUV transects that have been analyzed are presented here as examples of the capabilities and limitations of this technology.

Methods

The Seabed AUV is a stable platform that can be configured with a wide range of sensors including digital cameras and multi-beam sonars (Fig. 1). The AUV primary mission is to characterize seafloor benthic habitat by providing high resolution, color imagery in areas of steep and rugged terrain in combination with bathymetric and other oceanographic sensors. The Seabed is the only hover capable AUV that is able to conduct linear transects or detailed surveys of specific areas while maintaining a constant distance from the bottom. A Pixelfly 1024 x 1280 pixel resolution CCD camera, with 12 bits of dynamic range, is the primary optical imaging sensor. More information on Seabed components, sensors, control systems, and navigation can be found in Singh et al. (2004).

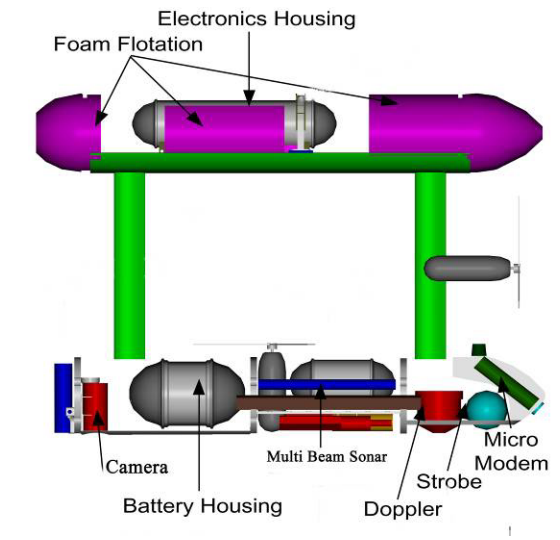

Figure 1. The Seabed AUV. Schematic diagram of the major components (top) and external view during deployment (bottom).

Seabed AUV high-resolution digital images, obtained in November 2004, were used to characterize the dominant components in two photo transects at La Parguera and Guánica, southwestern Puerto Rico. Each transect was approximately 400 m long and produced over 900 images, each measuring about 2.1 m wide by 2.0 m long. Every tenth image (n=90) along each transect were analyzed using 50 random points in the Coral Point Count with Excel extensions software (Kohler and Gill 2006). The dominant benthic categories were divided into: stony corals, gorgonians, sponges, algae/hardground, and unconsolidated sediments. The algae and hardground categories were combined since macro-algae and turf algae cover was extensive in hardground areas and the resolution of the images was inadequate for a more detailed analysis of these categories.

Results

Although the insular platform and shelf edge off La Parguera has the best developed reef system of the island, there is little information on the deeper reef zones in this area. The only published reports of the deep reef communities of La Parguera, to a depth of 125 m, revealed 25% coral cover at depths of 25-30 m but less than 2% coral cover at depths of 30-60 m (Armstrong et al. 2002; Singh et al. 2004).

The insular shelf in this area is 8-10 km wide with an average depth of 15-18 m from near shore to the shelf break (Morelock et al. 1977). The shelf edge has a buttressed-reef formation with channels that allow the movement of sand from the outer shelf to the upper insular slope, which descends at 43˚ in most places (Morelock et al. 1977). These authors report that there are no channels where the upper 30 to 40 m of the slope is vertical. The Parguera AUV transect started at 17°52.6' N, 60°2.6'W at a depth of 20 m and ended at depth of 94 m (Fig. 2).

The insular shelf south of Guánica Bay, located approximately 13 km to the east of La Parguera, is approximately 3-4 km wide and has average depth of 12 m. Extending southeast across the insular slope there is a submarine canyon that shoals to 12 m at the entrance to Guánica Bay (Morelock et al. 1994). The Guánica AUV transect started at 17°55.5' N, 66°52.3'W at a depth of 27 m and ended at a depth of approximately 130 meters (Fig. 3).

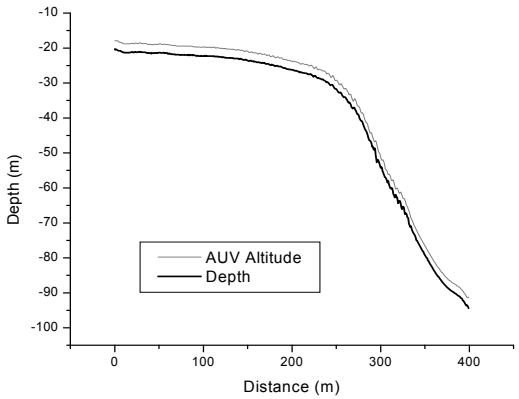

Figure 2. Depth profile and AUV altitude for La Parguera transect.

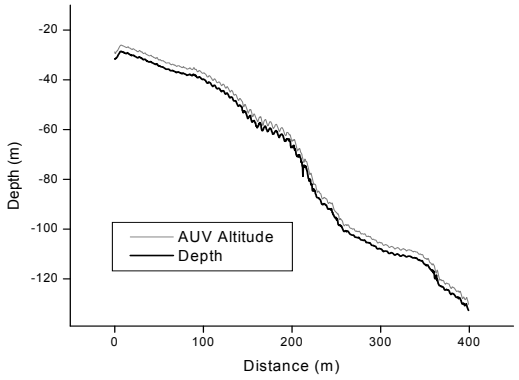

Figure 3. Depth profile and AUV altitude for the Guánica transect.

The La Parguera transect bathymetry reflects the geomorphology described by Morelock et al. (1977). The shelf-edge has a well-defined break from a gentle slope to one that is much steeper beyond the break, at a depth of approximately 30-40 meters (Fig. 2). The bathymetry at the Guánica transect (Fig. 3) is very different from the one observed in Parguera and can be described as a ramp. Ramps have no definite slope break and sediment facies grade evenly from sandier sediments associated with higher energy in shallower environments to muddier sediments in the low energy deeper water environment (Tucker et al. 1990). At both sites coral cover decreased with depth with the maximum percent cover of 7.8 and 4.0 for the Parguera and Guánica transects, respectively (Fig. 4). Notice that the 20-24 m depth interval was not present in Guánica and the 97-110 m depth zone was not sampled in La Parguera (Fig. 4, 6-9). The maximum coral cover in La Parguera was present at the shelf break, at a depth of 25-35 m and consisted of coral-sponge-gorgonian hardgrounds (Fig. 5a).

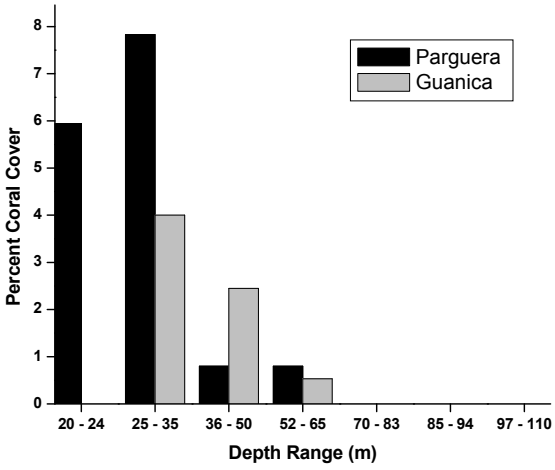

Figure 4. Percent coral cover by depth range for La Parguera and Guánica transects.

Similarly, the Guánica transect also lacked well-defined, structural coral reefs with hardgrounds also dominating the shallower geomorphology. Small isolated coral heads of the genus *Agaricia* were found to a depth of approximately 65 m in Guánica and to 87 m in La Parguera. Acevedo et al. (1989) reported that corals were present to a depth of 70 m in La Parguera.

The percent cover of gorgonians at all depths is more similar between the two transects with the highest values also present at the 25-35 m depth range (Fig. 6). After 65 m depth there is a noticeable decrease in gorgonian cover. Although sponge coverage was higher in La Parguera than in Guánica, dominance by sponges is apparent at both sites and

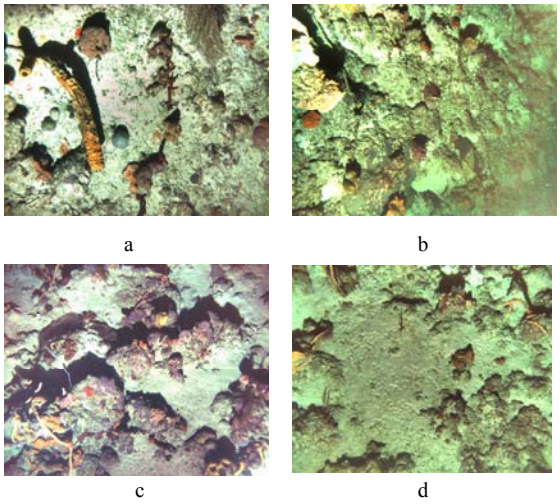

Figure 5. Hardground areas with isolated corals, sponges, and gorgonians dominated the two transects at the shallower to intermediate depths. AUV images from the La Parguera transect at 35 m (a), 67 m (b), 78 m (c), and 84 m (d) depths.

throughout all depths to 94 m (Fig. 7). Dominance by sponges, in the 30-100 m depth range, was also reported by Singh et al. (2004). Hardground areas with algae accounted for approximately 40% of the benthic cover in La Parguera and from about 40-70% in Guánica, both at depths less than 95-100 m (Fig. 8). The amount of unconsolidated sediment increased with depth, particularly at Guánica, where the highest amount of fine-grained sediments (96%) was found at the 97-110 m depth range (Fig. 9). A high degree of bioturbation at the deeper zones of both transects was evident from the AUV images (Fig. 5d).

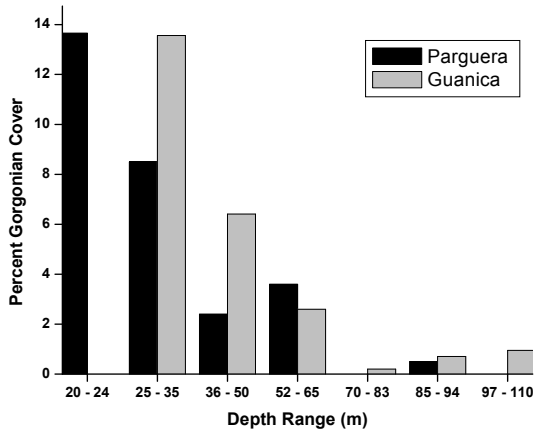

Figure 6. Percent gorgonian cover by depth range for La Parguera and Guánica transects.

Discussion

The high quality digital imagery provided by the Seabed AUV was used to describe and characterize the geomorphology and benthic habitats present to

depths of 110 m in southwestern Puerto Rico. These quantitative, geolocated AUV surveys also provide a baseline for future evaluation of change in the deeper coral reef zones.

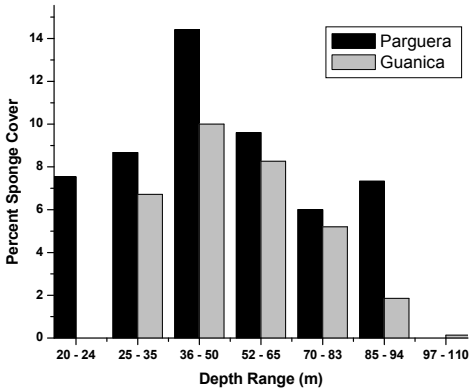

Figure 7. Percent sponge cover by depth range for both transects.

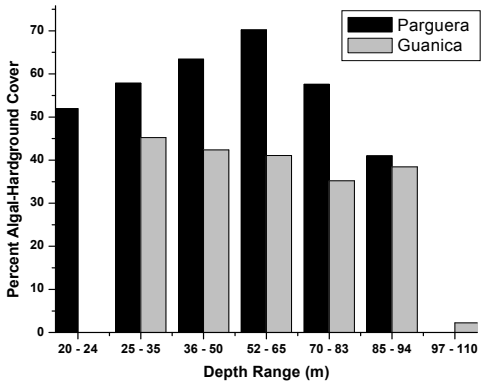

Figure 8. Percent algal-hardground cover by depth range for La Parguera and Guánica transects.

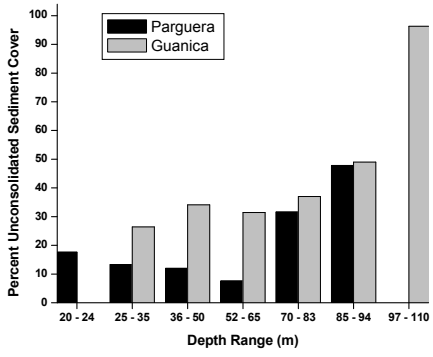

Figure 9. Percent unconsolidated sediment cover by depth range for La Parguera and Guánica transects.

Similar transects could also be used for large-scale mapping and monitoring of shallow (<20 m) reefs present in turbid insular shelf areas. In this case the AUV altitude from the substrate needs to be substantially reduced to minimize the light absorption of the water column allowing the capture of high contrast images. Besides benthic imaging, other sensors on the AUV such as fluorometers and turbidimeters can be used for monitoring changes in water quality parameters that are known to impact coral reef areas.

Reports on upper insular slope communities, from the shelf edge to 100 m depth, are scarce for the US Caribbean. Most of the research in this zone has been focused on fishery resources (Garcia-Sais 2005). The only published reports for Puerto Rico are from La Parguera, where coral cover was found to decrease from 24% at 25-30 m to less than 2% at 30-60 m (Armstrong et al. 2002; Singh et al. 2004). The results presented here from these two transects agree with the previous reports.

The depth limitations of conventional diving have left mesophotic reefs largely unexplored. Even though technical diving has extended the depth range of diving surveys, the large-scale assessments and monitoring of mesophotic reefs by divers remain impractical. The Seabed AUV has the distinct advantage of allowing frequent access to large areas of mesophotic reefs with an endurance of up to 8 hrs producing transects that could be several kilometers in length. In addition, data from Seabed AUV sensors and related imaging technologies can be used to conduct multi-beam sonar surveys, photo mosaicking, and multisensor fusion of acoustic and optical data.

Although the AUV uses a high resolution and dynamic range camera, the relatively high altitude of the vehicle from the bottom makes identification of certain algae, sponges, disease and other substrate types difficult. A comparison between the AUV and a diver-held video camera method showed similar percent cover values for scleractinian corals but increasing variation in other categories such as macroalgae, coralline algae, gorgonians, and sponges (Nemeth et al. 2009). The diver held video camera provided closer images which enhanced the ability to identify benthic organisms but greatly underestimated the percent cover of gorgonians (Nemeth et al. 2009). Also, detection of coral disease was most effective with direct visual assessment of individual coral colonies by divers.

The large-scale characterization and monitoring of coral reefs in optically-deep waters requires the use of *in situ* platforms equipped with optical and acoustic sensors. Optically-deep waters are present over large coastal areas of Puerto Rico where coral reefs are known to exist. Approximately 43% of the potential

reef habitat within the Puerto Rico-USVI insular shelf and slope is found between 30-100 m. The Seabed AUV has proven to be ideally suited for mapping and monitoring mesophotic reefs, which for most areas of the world remain largely unknown.

Acknowledgments

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GIS derived spatial analysis as a tool to predict nearshore coral reef fish species presence in American Samoa

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Abstract. The U.S. National Park Service has undertaken a monumental task of inventorying species found within park boundaries. This is part of the Inventory & Monitoring program aimed to increase our management efforts in all the U.S. National Parks. In the National Park of American Samoa, the inventory of reef fish has helped to discover 47 new species records for the territory of American Samoa. This was done using both GIS polygons derived from known geographic distributions of marine fish as well as in-water surveys using SCUBA and technical diving procedures. Using ArcInfo GIS software, a distribution map was created for all known coral reef fish species from the central South Pacific. GIS benthic habitat maps were then used to locate suitable and likely locations for a given species. In-water surveys were then conducted to look for the predicted species. Using this technique 138 additional species were presumed to be located in American Samoa. To date the in-water surveys have found 47 new records, of these, 37 were assumed present from the GIS modeling but had never previously been found. Many of the remaining species are small and cryptic and are unlikely to be encountered without the use of ichthyocides.

Key words: GIS, predictive mapping, Samoa, coral reef, fish

Introduction

Though we often idealize the South Pacific as pristine and untouched, in reality many of the small tropical islands of the South Pacific have had significant alterations and anthropogenic influences. Increasing negative impacts on coral reef habitat and species abundance have been correlated with their proximity to greater population densities (Brainard et al 2008). As a result many governments, agencies, and private organizations have begun initiatives to identify, prioritize, and delineate areas of special importance so that we may mitigate anthropogenic influences (Pittman et al 2007, Allen 2008, Ward et al 1999).

In the United States one of the federal agencies that has begun this process is the U.S. National Park Service. It has undertaken the monumental task of inventorying and monitoring all species found within park boundaries both below water and on land. This is part of the Inventory & Monitoring program aimed at increasing management capabilities in all U.S. National Parks. As a part of this effort, a new fish species list for American Samoa was deemed necessary. Previous to this study the most recent list of fish presence data came from work completed 25 years ago. Researcher inventories, species lists, and museum collection records for Samoa were analyzed and it was noticed that they all emanated from similar, if not identical, collection locations and often from similar depth strata. It was then hypothesized that key habitats and various depth profiles may have been undersampled and overlooked during these previous efforts.

Predictive mapping for species presence and abundance is relatively well developed and routinely used in terrestrial environments (Pittman et al 2007, Miller et al 2004, Guisan and Zimmerman 2000). Within the marine environment much less predictive mapping has been done until very recently (Pittman et al 2007, Friedlander et al 2007, Spens et al 2007). Many of these studies are theoretically based, using computer models and statistical variables to ascertain species data and few have little if any "groundtruthing" of the predicted species presence. In this study, we make fish species presence lists based on predictive modeling using GIS based methodologies as well as in water observations. We sought to sample both historical collection sites as well as additional undersampled areas based on habitat and depth to look for new species and new records of nearshore coral reef fish species.

Material and Methods

The study area used for this research was the nearshore waters of Tutuila, American Samoa (14°S, 170°W) a U.S. territory in the South Pacific (Fig. 1). Tutuila, the largest island in the territory, exhibits

more diversified habitat types (coral reefs, lagoon, sand, mangroves, etc.) with more varied depths and bathymetric complexity than the other islands in the archipelago due mostly to its much greater size (Brainard et al 2008). Given the diversity of available habitat types, the broadest range of fish species should occur around this island.

Figure 1: Study site location, Tutuila, American Samoa.

Adapting the methodology described by Allen (2008), known point collection records (California Academy of Sciences, Australian Museum, Western Australia Museum, and the Bishop Museum) and verified field sightings data (various colleagues and NOAA-CRED) were used to analyze distribution patterns of nearly 3,000 nearshore coral reef fishes throughout the Pacific using GIS mapping software (ESRI, ArcMap 9.2). All pertinent collection information such as benthic habitat and cover types was reviewed. From these point data, polygons were created to connect points into a generalized equal area range map for each species (Fig. 2).

Figure 2: A representative equal area range map, in this case for *Iniistius pavo*, used for nearshore coral reef fish species presence predictions.

Equal area range maps were utilized in order to represent a true geographic relationship between collection points and theoretical range maps. It is very likely that there is a wider dispersal zone for each species around a given collection point, but for added conservativism, only exact locations were used to derive polygons without any type of buffer zone around individual point data. These range map polygons for individual species cover vast areas of the Pacific, most of which is deepsea/pelagic habitat. The distribution of individual reef species is assumed only to occur in areas with suitable habitat (e.g. nearshore shallow-water coral reefs and associated habitats). Species that were already known to occur (Wass 1984), were then removed from the dataset and the remaining species were examined. There were 138 species not previously recorded that had predicted ranges that included American Samoa or came within 400km of the Samoan Archipelago.

Using existing natural history data, habitat preferences for these species were identified. Taking species thought to occur in the territory from the data provided in the range maps, predictive habitat maps were then created for each of these species and their likely locations plotted using specific habitat parameters. Habitat parameters were derived for each species from the published literature utilizing known natural history and habitat useage (e.g. *Acanthurus olivaceus* occurs over coral and rubble bottoms, *Acanthurus lineatus* is most frequently encountered on shallow reef flats, while *Acanthurus thompsoni* is found near steep drop-offs). Habitat maps were created using orothorectified aerial photographs as well as IKONOS and Quickbird satellite data, following methodology developed by NOAA (Coyne et al 2003). The panchromatic visual imagery was first interpreted and reef zones delineated to classify major sub-types of reef (reef flat, slope, backreef, crest, and lagoon). Next Quickbird and IKONOS multispectral color imagery was used to delineate the nearshore area into broad classes of habitat (coral reef, plant, rock, sand). Each of these was then subdivided into narrower categories (i.e. emergent vegetation, macroalgae, seagrass, etc.). Output from the process of delineating the broad reef zones and habitat classes and their corresponding subclasses was a series of digital GIS layers. These polygons were then overlaid to produce a highly accurate and delineated thematic map of the nearshore benthic habitat. The overlay process involved adjusting the relative opacity for each individual map and then projecting each into the same plane so that multiple features could be viewed simultaneously. It was determined that an intersect operation did not identify subtle differences in the various layers and was therefore carried out visually to ensure accuracy of the interpretation. This process was repeated for each of the 138 predicted fish species. For example, *Iniistius pavo*, is known to occur at depths from 20-100m in areas with high sand near coral reefs with high rugosity. Bathymetric data

collected by the NOAA coral reef ecosystem division (Brainard et al 2008) was used to stratify the available habitats by depth. Given the size of the study location groundtruthing was not possible at all locations, therefore random sites were chosen and grountruthed using scuba. An accuracy rate of >90% was found at each of the 15 random sites. Exploratory dives were then made during the day and night into those likely habitats to look for the presence of predicted fish species. Additional survey dives were also carried out at historical collection sites and at various depths in these locations.

Results

Many fish species were found in American Samoa that were previously unrecorded. Most of these fish were species that are small, cryptic and/or nocturnal. There were however several species, including two stingrays, which attain significant sizes of 1m or more. These rays were found in sand flat habitats that have historically been undersampled (Fig. 3). Of the 138 fish predicted that had not been previously seen in American Samoa, 37 were located during this study, including one new family record. A further 10 fish species were located during the in-water survey dives which were unexpected (e.g. not predicted by the mapping procedures and previously unrecorded from Samoa). Of the 37 new species records, 34 were located in habitats previously poorly documented or searched. At least 3 species have been discovered which may be new to science including a Pomacentrid, Apogonid, and a Gobiid.

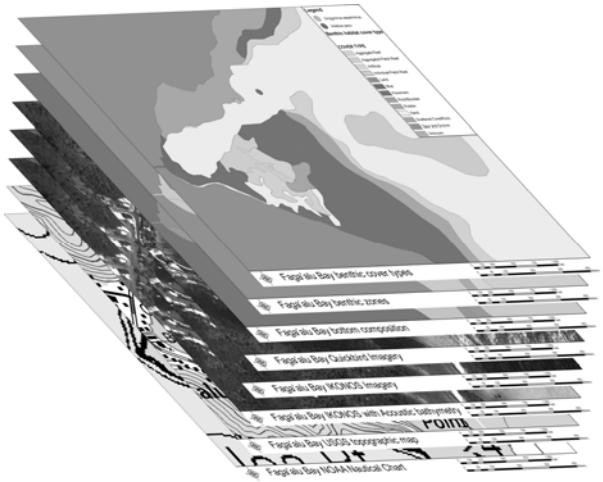

Figure 3: Multiple layers of benthic habitat maps, bathymetric data, and satellite imagery used to predict locations of predicted fish species.

Discussion

Many researchers, like recreational divers, often go to established sites. Given the limited time often available for studies, maximizing results by going to known locations is often prudent. This approach does however ignore certain habitats that may then get undersampled, if at all. Part of this study was focused on looking in those locations other researchers have deemed unfit or unworthy of limited research time. For example, Faga'alu Bay, within Pago Pago Harbor is often considered too degraded and impacted to be of any scientific value. Yet it is within this bay that many of the new species records were found.

Some new species records, like the two stingray species, are large and non-cryptic in nature and thus it is unlikely that they were present in previous years surveys and seem to indicate a recent colonization. While many species which were predicted were discovered, several others were found that were unexpected. This emphasizes the need for groundtruthing, as this was an important component of this study, not only to validate benthic habitat maps, but also to verify theoretical species presence and more significantly yielded 10 new unexpected records.

Given the small size, cryptic nature, and often nocturnal habits of the remaining predicted fish species, it is unlikely that using non-destructive sampling methods like those employed in the present study will uncover many additional species records from the predicted list. This study shows that using GIS based predictive modeling and non-destructive methods in novel habitats can yield advances in our knowledge of fish species in remote locations.

Acknowledgement

This project would not have been possible without the cooperation and data from various agencies; USFWS, USGS, NPS, NOAA Coral Reef Ecosystem Division, NOAA Biogeography, California Academy of Sciences, Australian Museum, Western Australia Museum, and the Bishop Museum.

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An investigation of coral reef fish assemblage modelling with geostatistical methods

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Abstract. The objective of this study was to determine the spatial distribution of reef fish assemblages according to the habitat types of the Coral Coast, Fiji by using geostatistical methods. The study focused on the reef flat zone covering 45 km² which was divided into 22 geomorphological reef units. Substrate and fish data were obtained from a previous field survey of 311 transects. We worked with five classes of habitats (sand, rubble, macroalgae, seagrass and coral) which were defined according to thresholds of 20% for the biotic substrate and 50% for the abiotic. Nine fish families were selected due to their importance for the fisheries and as reef health indicators. We conducted batches of multivariate statistical tests to identify significant patterns of fish assemblage distribution. Prediction maps of fish abundance were created by using the co-Kriging geostatistical model. Results showed that sand, seagrass and coral habitats hosted significantly different assemblages. The quality of the predictive models varied highly depending on the location and the fish family of which Acanthuridae and Scaridae showed the best accuracy. Reef fish modelling using a geostatistical approach provided encouraging results considering that this investigation was conducted with previously collected data and within a reduced resource context.

Key words: coral reef fish, assemblage, habitat, geostatistical modelling, Fiji

Introduction

Coral reef fish communities are influenced by the distribution and abundance of component species. Their distribution and abundance are in turn influenced by the interaction between their behaviour and the physical and biological environment. Many studies have shown that reef fish abundance and species richness are correlated with the biological nature of the substratum and notably with the extent of live coral cover (Bell and Galzin 1984; Chabanet et al. 1997; Garpe and Ohman 2003; Bouchon-Navaroa et al. 2005). However, habitat composition has not always been found as the main factor explaining fish community structures (Roberts and Ormond 1987; McClanahan and Arthur 2001; Dominici-Arosemena and Wolff 2005).

Although spatial models have been widely developed for terrestrial environments, they have just been recently applied to the marine ecosystems (Pittman et al. 2007). Most of the coral reef studies involving spatial modelling have mainly focused on assessing the effect of marine reserves on single fish species or assemblages (Núñez-Lara et al. 2005) and on improving the ecological knowledge of fish populations to support ecosystem-based management (Mellin et al. 2007; Pittman et al. 2007; Mumby 2006). As collecting data on extensive areas is regarded as too expensive and time costly, ecologists are now focusing on predictive methods and their improvement. Extrapolation is one of the approaches to predict patterns, where known data are projected, extended or expanded into an area not sampled based on assumptions of continuity and correlation among the variables (Miller et al. 2004).

Coral reefs are of crucial importance for the people of the Pacific Islands Countries since they support numerous economic and cultural activities in addition to providing environmental services. The Fiji Islands have been the location of many marine survey programmes providing a large quantity of data on reef benthic and fish assemblages. However, spatial analysis has been rarely if ever conducted and normal analysis has not been exhaustively carried out.

The main aim of this study is to model the spatial distribution of the reef fish assemblages according to the habitat types of the Coral Coast, Fiji Islands using geostatistical methods. The influence of the benthic composition on family taxa abundance is first assessed and then the feasibility of modelling reef fish distribution with the ArcGIS Geostatistical Analyst extension is investigated. Considering the Fiji context, where scientific resources are limited, this paper illustrates an experimental approach for coral reef research.

Material and Methods

Study Site

This study focused on the Coral Coast region located along the southwest coast of Viti Levu, Fiji's main island (Figure 1a). The Coral Coast is

bordered by the longest fringing reef found within Fiji's waters, stretching along about 80 km with a seaward extension of 500 m to 1 km. The reef system is made of 22 geomorphological reef units separated by channels.

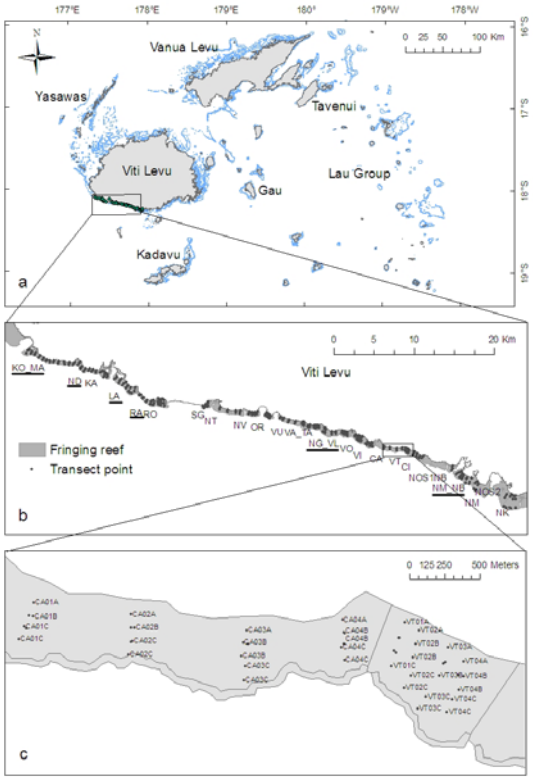

Figure 1: Study site. a) the Fiji Islands; b) the Coral Coast fringing reef and the sampling sites surveyed by CCC. Codes refer to the reef unit names. Underlined reef units were selected for modelling; c) close up showing the sampling design. Two points correspond to the start and end points of one 100 m section (named A, B or C).

The human population along the Coral Coast is distributed into villages of about 100 to 300 inhabitants with the exception of Sigatoka town where about 8,440 people live (Fiji Islands Bureau of Statistics 2007). With development and increasing population, the main threats to the ecosystem and its resources have been identified as being the overexploitation of fish and corals, inefficient waste management leading to coastal water pollution, and erosion and siltation due to inland deforestation (Thaman and Aalbersberg 2004).

Secondary Data and Benthic Habitat Classification

The field data used for this study were provided and collected by Coral Cay Conservation (CCC) between July 2004 and May 2005 (Rowlands et al. 2005) (Figure 1b-c). Data were collected along transects located perpendicular to the shoreline on the reef flat zone. Each transect was divided into three 100 m long sections which were laid down within three different zones of exposure to offshore influence. A total of 311 sections were surveyed by snorkelling based on a modified Reef Check technique. Assessment of benthic coverage was performed following the point intercept method along a 100 m tape. Substrate type was recorded every 0.5 m, providing a total of 200 data points per 100 m section. Fishes were counted along the same 100 m tape using the belt transect methodology applied within a 5 m wide by 20 m high box.

A hierarchical classification scheme of habitats was established *a priori* based on previous works and on expert knowledge. The classification scheme comprised of five habitat classes: sand, rubble, macroalgae, seagrass and coral. A simple quantitative component was incorporated with arbitrary percentage cover thresholds. Biotic substrates (i.e., coral, seagrass and macroalgae) were considered dominant when covering 20% of a transect, whereas a threshold of 50% was assigned to the abiotic categories (i.e., sand and rubble).

Multivariate Analysis

Multivariate statistical analyses were first conducted to identify significant patterns of fish assemblage distribution. Nine fish families (Chaetodontidae, Lethrinidae, Mullidae, Serranidae, Scaridae, Siganidae, Lutjanidae, Acanthuridae, Labridae) were studied due to their importance in terms of resources for the communities and their ecosystem health indicator status. Analyses were performed using the routines provided by the PRIMER v6 software (Clarke and Warwick 2001). Comparison of fish assemblage structures was based on the Bray-Curtis similarity coefficient. Investigation of the effect of habitat type on fish family abundance was done with the ANOSIM (ANalysis Of SIMilarity) significance test. To identify the characteristic families of each habitat type SIMPER (SIMilarity PERcentage) analysis was applied to the data, allowing identification of typical variables through calculating their average contribution to the intra-group similarity and to the inter-group dissimilarity.

Spatial Modelling

Modelling was based on the results of the multivariate analysis. Prediction maps of fish abundance were created at the reef unit scale, using the co-Kriging geostatistical model. The extrapolation is based on a trend identified from the data and the spatial autocorrelation theory assuming that features close to each other are likely to be more similar than the ones apart from each other. Co-Kriging is a stochastic model which provides an estimation of the variance for any point predicted. It is thus possible to quantify the quality of the surface models by measuring the statistical error of predicted surfaces. In addition, the co-Kriging model allows multivariate analysis where the predicted values are estimated according to the correlation with an influencing variable.

Several extrapolation models were tested by setting different parameters, such as the lag number and size, or the searching neighbourhood shape and methods (Johnson et al. 2001). They were compared using the cross-validation results. This provided information on the statistical performance of the model in predicting fish abundance values. A model was qualified as good if the mean prediction error is close to zero, the root-mean-square standardized prediction error is close to one and the root-mean-square prediction error is small. These measurements indicate, respectively, unbiased predictions, accurate standard errors and predicted values similar to the measured values (Johnson et al. 2001). The models with the lowest statistical errors obtained for each family and reef unit were then selected and the surface maps were converted into polygon layers.

Results

Fish-Habitat Associations

Exploratory analyses and statistical tests showed that fish assemblages were structured differently among different habitat types (Table 1). Differences were highly significant for macroalgae vs. sand (p=0.001), seagrass vs. coral (p=0.001) and rubble vs. sand (p=0.003). In addition, fish assemblages found within coral and within rubble habitats had significantly different structures compared to those associated with sand and seagrass dominated areas respectively.

Table 1: Results of the ANOSIM analysis. Global R=0.116 and p=0.001. Confidence intervals are represented by asterisks as follow: * 95%; ** 99.9%. Only significant results are shown here.

Pair-wise tests	R value	p value
macroalgae vs. sand	0.46	0.001**
seagrass vs. coral	0.558	0.001**
rubble vs. sand	0.291	0.003**
coral vs. sand	0.182	0.026*
rubble vs. seagrass	0.169	0.044*

According to the SIMPER analysis, the average contribution of the family to the intra-group similarity showed two patterns of association family-habitat (Figure 2). Labridae contributed at 95% to the similarity within the seagrass group and was thus considered as a discriminating family. In addition, given their high percentage contributions, Scaridae and Acanthuridae can be considered as discriminating families of the groups seagrass vs. coral and coral vs. sand.

Labridae had the highest contribution to the inter-group dissimilarity and the order of percentage contribution of the families was the same in all of the pair-wise results (Table 2). Dissimilarity of fish assemblages between macroalgae and sand habitats was due to the high abundance of Labridae in sand areas. Three pair-wise tests (macroalgae vs. sand; rubble vs. sand; rubble vs. seagrass) showed that the contribution to the dissimilarity was dominated by Labridae while in the two others (seagrass vs. coral; coral vs. sand), the contribution were more evenly distributed among Labridae, Scaridae and Acanthuridae families. Given their high percentage contributions, Scaridae and Acanthuridae can be considered as discriminating families of the groups seagrass vs. coral and coral vs. sand.

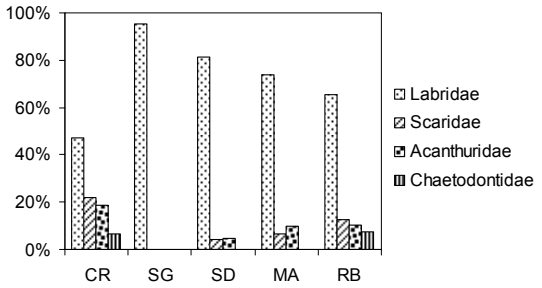

Figure 2: Average contribution of the fish families to the intra-group (habitat type) similarity. CR: coral; SG: seagrass; SD: sand; MA: macroalgae; RB: rubble

Spatial Prediction of Fish Abundance

Surface predictions of abundance were created for the three families which displayed the strongest patterns in their relationship with habitat types: Labridae, Acanthuridae and Scaridae. Labridae distribution was predicted at two reef units, NM_NB and RA (Figure 1), where seagrass habitats have been mapped. Modelling of Acanthuridae and Scaridae abundance was performed based on coral cover and thus at four reef units (KO_MA, LA, ND and NG_VL). Models including data on the three biotic habitats (seagrass, macroalgae and coral) were also developed in order to investigate whether considering additional habitat characteristics improved the results.

Table 2: Percentage contribution of family to assemblage dissimilarity between habitat types.

Family	rubble vs. seagrass	seagrass vs. coral	macroalgae vs. sand	rubble vs. sand	coral vs. Sand
Labridae	45.21	30.96	52.57	48.07	37.75
Scaridae	19.96	26.54	14.93	19.55	24.32
Acanthuridae	14.07	23.73	13.5	12.94	20.41
Chaetodontidae	7.49	6.84	7.42	7.94	6.29
Siganidae	6.15	4.33	5.6	4.91	3.98

Table 3: Results of the cross-validations of the fish abundance models which showed the lowest statistical errors. Models based on "3 habitats" included seagrass, macroalgae and coral covers.

Model	Reef unit	Mean error	Root-mean-square	Average standard error	Mean standardized	Root-mean-square standardized
Labridae + Seagrass	RA	0.236	63.39	62.76	0.006	1.018
Acanthuridae + 3 habitats	ND	0.008	22.18	23.08	0.001	0.962
Scaridae + 3 habitats	ND	0.055	17.65	17.96	0.002	0.987

Comparison between the models using cross-validation showed that the quality of the models was highly variable among families and reef units (Table 3). Surface prediction of Labridae abundance appeared to be more difficult to create with high accuracy since the models were not satisfactory with mean prediction errors ranging from -1.546 to 0.236. However, prediction was best fitted at RA when only seagrass was included in the model.

Models of Acanthuridae and Scaridae seemed to provide better results at some locations. The best mean prediction errors were for both families found at ND. Considering the models of Acanthuridae, the best model predicted abundance with an average standard error of 22 at ND. This uncertainty appeared to be overestimated since the root-mean-square standardized error was less than one (0.962). It seemed that the model quality was slightly improved with the integration of the three biotic habitat types. Mean prediction error of Scaridae abundance was lowest at ND with the coral based model. However, Scaridae abundance was estimated with an average standard error of 18 at ND based on the three habitat covers. The root-mean-square standardized error was 0.987 indicating a better assessment of the variability though there remained an apparent overestimation of abundance. Cross-validation results showed that Acanthuridae and Scaridae predictions were of better quality when they were based on the three-habitat model while Labridae model was best fitted with the one-habitat model.

Discussion

The modelling of selected fish families using geostatistical methods provided contrasting results depending on the site and the families. It appeared that models were likely to create accurate surface predictions of abundance at one specific reef unit (ND) for one family (Acanthuridae). It is possible that the level of spatial autocorrelation in reef fish distribution was too low to provide accurate models. Reef fish distribution is influenced by spatial, temporal and behavioural factors which might have also affected the level of accuracy. In addition, the number of samples was a constraint preventing the modelling of fish abundance at several reef units. It suggests that additional field data may improve the model accuracy but it compromises the intent of the method which is to obtain results with already available data. Prediction maps of Scaridae and Acanthuridae showed a narrow range of abundance variation across the reef unit (Figure 3). Such patterns are unlikely to occur in reality and when the predicted values were compared with the measured ones, the variation was not reflected within the former. However, the results need to be compared with the reality of the field.

It has been argued that the tendency of the fish species to move and migrate influences the results of spatial variation models (Ault and Johnson 1998). They found that the best predictive models of fish density occurred for site-attached species such as the members of the Pomacentridae (Damselfish) family.

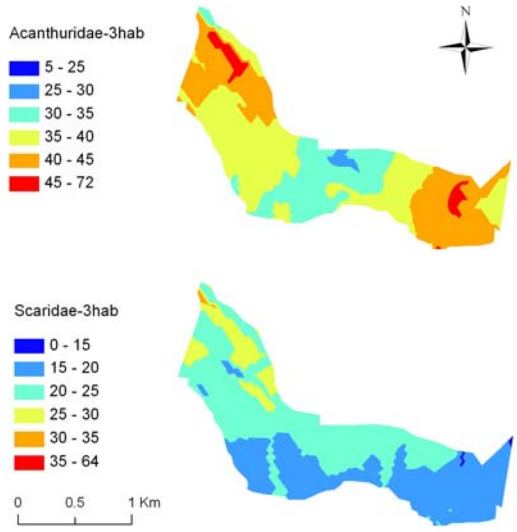

Figure 3: Surface predictions produced by the two statistically best co-Kriging models of the Scaridae and Acanthuridae abundances at the ND reef unit.

Reef ecologists first conducted their research with the notion that fish communities were organized in an equilibrium system to later shift toward an approach which comprehends reef fish communities as an open non-equilibrium system (Sale 1991). Owing to the difficulty in drawing general patterns in reef fish community organization, ecologists have for some time accepted that reef fish assemblages were driven by

stochastic processes. Recent research has increasingly shown that the habitat utilization by reef fishes was not a random process (Ault and Johnson 1998; Friedlander et al. 2007; Garpe and Ohman 2007).

If reef fish assemblages are non-equilibrium systems which develop within a dynamic environment, their modelling can be done through simulation models (Guisan and Zimmermann 2000). However, this needs a high level of understanding of the relationship between assemblage and habitat. For instance, spatial extrapolation methods encompass the determination of the scale effect on the response variable, in this case on the fish assemblage structures (Miller et al. 2004). It thus appears necessary to gain a good understanding of the scale-dependence of the reef fish assemblages in order to reach accurate predictions. Yet again it implies a design of sampling methodologies allowing the estimation of the scale effect on the results.

Modelling of reef fish assemblages using geostatistics showed some satisfactory results in selected taxa and defined geographic areas. However, the outcomes of this investigation suggested that geostatistics applied to reef fish ecology could provide better results if used to define sampling design for a non-parametric spatial approach. In addition, there is a need to deepen the investigation of the Coral Coast reef fish assemblage patterns and processes. By examining the impacts of other influencing factors such as the habitat complexity and human pressure, the model results could be improved. Integrating human pressure could be based on the fishing pressure gradients documented by previous studies done in Fiji (Jennings and Polunin 1995; Dulvy et al. 2004). Study on the temporal variation and the recruitment effects would be of great interest but would be more costly in terms of time and funds. Similarly, the model may be improved by basing it at the species level which would allow the examination of other indicators such as species richness, biomass and more clearly defined feeding, ecological and behavioural guilds.

This investigation provided encouraging results considering that it was conducted with previously collected data and considering the Fiji context where resources are limited. Moreover, it demonstrated to local scientists and practitioners the potential of Geographic Information System and spatial analysis to coral reef science and management.

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Changes in spectral reflectance in response to salinity variation in *Siderastrea radians* from Florida Bay, Florida USA

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Abstract. Spectral reflectance (*R*) of coral reefs is an apparent optical property that is widely used to distinguish these communities in remote sensing assessments. In this study *R*'s of *Sidereastrea radians* collected from five basins in Florida Bay were measured at the termination of a 2-week salinity (30, 20, 15, 10) versus population mesocosm experiment. The five basin populations represented a spatial and freshwater-land influence gradient from northeast (estuarine-to-hypersaline) to southwest (marine) Florida Bay. Spectral reflectances generally increased with decreased salinities with the greatest increases occurring in the more marine populations. The marine populations also exhibited the "brown" reflectance spectra mode while the more estuarine populations exhibited predominantly "blue" reflectance spectra. Two of the interior basin populations exhibited spectral shifts from "blue" to "brown" in response to low salinity treatments. Non-metric multidimensional scaling analyses indicated stronger salinity-based versus population-based clustering of the reflectance spectra. Thus, while coral *R* may be independent of taxonomic or geographic differences, reduced salinity does affect this parameter in *S. radians* from this marginal coral habitat.

Key words: Spectral reflectance, salinity, *Siderastrea*.

Introduction

The health of corals may be assessed using a number of metrics (i.e., growth, reproduction, metabolism, etc.), but loss of zooxanthellae or their pigments is the only remotely detectable measure of coral health because of the resulting changes in their spectral reflectance (Holden and LeDrew 1998, 2001). Spectral reflectance (*R*) of corals is an apparent optical property that is also widely used to distinguish these communities and monitor their status in remote sensing assessments (Green et al. 1996; Karpouzli et al. 2004). The magnitude and shape of coral *R* spectra are generally distinct from other benthic communities and bare sediments (Hochberg and Atkinson 2000), but independent of within-coral geographic and taxonomic differences (Hochberg et al. 2004). There are generally two spectral shapes of coral *R*, the "brown" mode, which is determined by zoothanthellae pigment absorption, and the "blue" mode, which includes expression of a coral host pigment. Reflectance spectra of stressed or bleached corals are generally higher and optically different than those of healthy corals, due to the loss of zooxanthellae pigments (Holden and LeDrew 1998).

Siderastrea radians (Pallas) Blainville is a hardy coral found throughout the tropical western to eastern Atlantic and Caribbean region that inhabits so-called marginal habitats away from characteristic reef communities (Veron 2000). In Florida Bay, this species occurs in areas of little sediment and low abundance of seagrasses (Figure 1). Concern over the long-term health of the Florida Bay ecosystem and the adjacent Everglades led to the Comprehensive Everglades Restoration Plan (CERP). One of the primary goals of CERP is to restore estuarine conditions in the Bay by increasing the quantity and quality of freshwater entering Florida Bay, which will lower salininties. The effects of reduced salinity on coral communities within Florida Bay have largely not been considered. Coral stress or bleaching may occur in response to decreases in salinity (Hoegh-Guldberg 1999), thereby affecting *R*.

In this study *R*'s of *Sidereastrea radians* collected from five basins in Florida Bay were measured at the termination of a 2-week salinity (30, 20, 15, 10) versus population mesocosm experiment designed to examine the salinity tolerance of this species. The five basin populations examined represented a spatial and freshwater-land influence gradient from northeast (estuarine-to-hypersaline) to southwest (marine) Florida Bay (Figure 1). The experimental salinities were chosen to encompass the range of salinities that occur across these five basins.

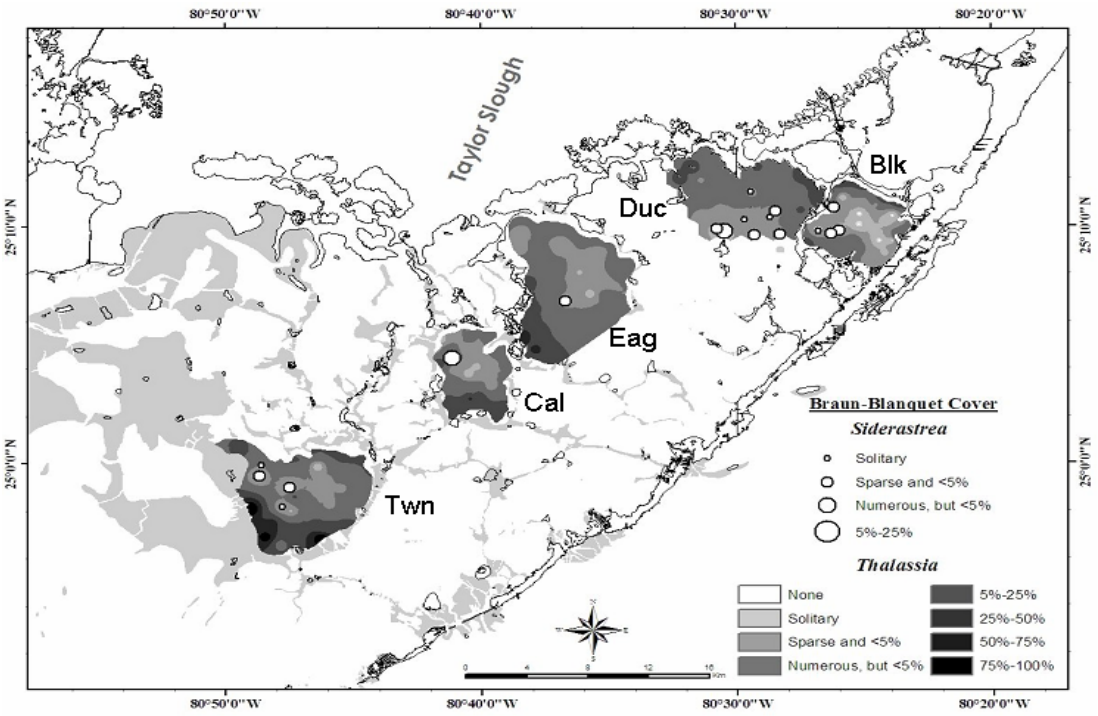

Figure 1 Distribution of *Siderastrea radians* and *Thalassia testudinum* in Florida Bay.

Materials and Methods

During Fall 2006 Fish Habitat Assessment Program (FHAP) field surveys, sixteen *S. radians* colonies (≤3-5 cm in diameter) were collected per basin from locations where the highest colony densities were previously observed. The five sampled basins, from northeast to southwest, were Blackwater Sound (Blk), Duck Key (Duc), Eagle Key (Eag), Calusa Key (Cal) and Twin Key (Twn) Basins (see Figure 1). Colonies were transported to the greenhouse at the University of North Carolina Wilmington, Center for Marine Science (CMS), Wilmington, NC overnight. Care was taken to ensure colonies were never removed from seawater and that temperatures and salinity were maintained under near-ambient field conditions.

Colonies were placed in 40-1 aquaria filled with high quality seawater and containing gravel-bed filter aeration systems. One colony per basin was randomly chosen and placed in each of 16 aquaria. Location was randomly assigned yet placement ensured that a colony from each basin was in each tank position at least twice. After one week acclimation at a salinity of 30 (the average salinity ±1 at collection sites), target treatment salinities of 10, 15, 20, and 30 (controls) were assigned to each of 16 aquaria (n=4, level of replication is the tank). Salinities were decreased daily by adding distilled water to experimental tanks to drop salinity at a rate of 2 day^{-1}. This rate of change was chosen to represent a realistic drop in salinity in the Bay during flushing and/or rainfall events (Kahn and Durako 2005, 2006). We measured *R* of the dorsal surface for all coral colonies 4 days after the lowest salinity treatment target was reached.

Reflectance (bi-directional) is the fraction (%) of downwelling radiance that reflects upward as a function of wavelength (λ) [$R(\lambda) = L_u(\lambda)/L_d(\lambda)$]. Radiance measurements employed an Ocean Optics USB2000 fiber optic spectrometer coupled to a 400 μm diameter UV-VIS reflection probe (Ocean Optics QR400-7-UV-VIS). To measure upwelling radiance [Lu(λ)], the probe was positioned 1 cm above the coral dorsal surface at a 45^o zenith angle (Hochberg and Atkinson 2000). Immediately following, downwelling radiance [Ld(λ)] was measured using a Spectralon diffuse reflectance target (Ocean Optics WS-1) placed adjacent to, and in the same plane as, the coral. It was assumed that the Spectralon target is Lambertian (i.e., reflects light equally in all directions).

Results

Reflectance spectra for the control treatment corals (salinity 30) exhibited a brown mode triple-peaked pattern (570, 600 and 650 nm) for the Blackwater Sound, Calusa Key and Twin Key Basin populations and a blue mode plateau between 600 and 650 nm in the more interior Duck and Eagle Key Basin populations (Figure 2, *sensu* Hochberg et al. 2003). Spectral reflectances generally changed little or increased slightly at the decreased salinity of 20, with

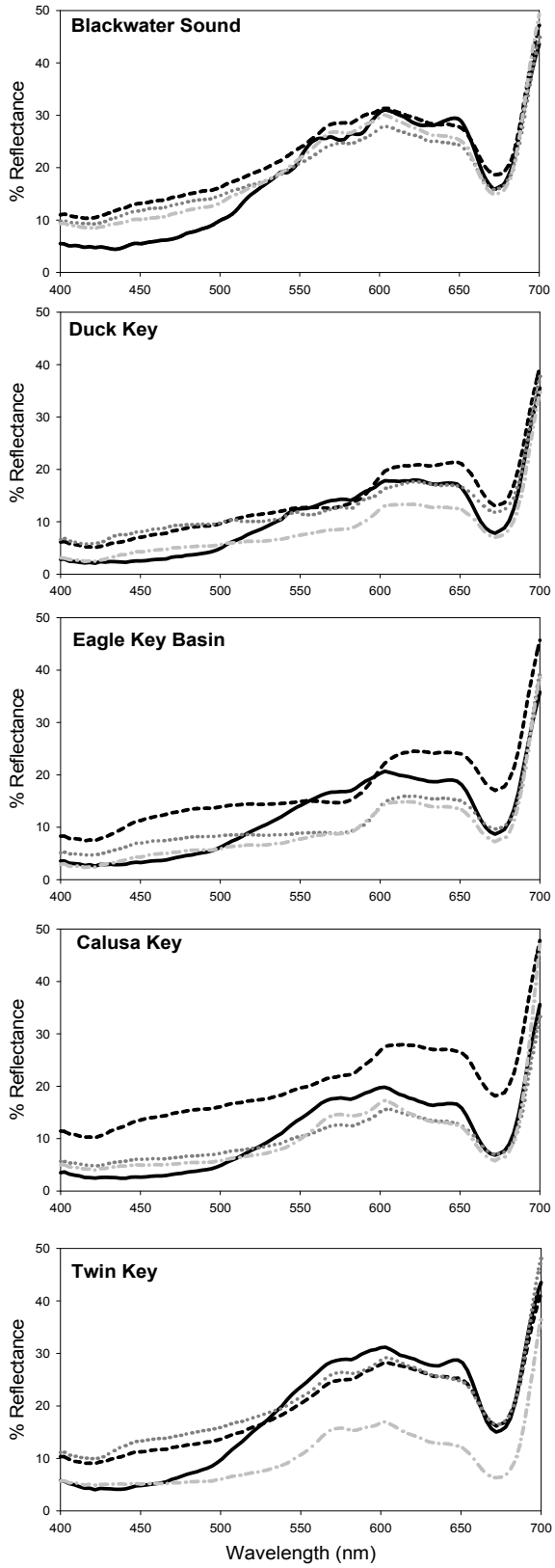

Figure 2. Reflectance spectra of *S. radians* from 5 Florida Bay basins in four salinity (10=black solid, 15= black dash, 20=grey dots, 30=grey dot-dash) treatments (n=4).

the greater increases in *R* occurring in the 15 and 10 salinity treatments. At the lowest treatment salinity (10), reflectance patterns were quite distinctive spectrally and exhibited differing population patterns. For all five populations, reflectance was generally lowest in the salinity 10 treatment at wavelengths <500 nm, but was highest at wavelengths >500nm for the most marine populations (Twin Key Basin). *R* was relatively high across PAR in the salinity 15 treatment for the three interior basin populations (Duc, Eag, and Cal, Fig. 2). Two of the estuarine basin populations (Eagle & Calusa) exhibited spectral shifts from blue mode to brown mode *R* in response to changes in salinity.

Non-metric multidimensional scaling analyses indicated that reflectance spectra exhibited similarities based on treatment salinity, with high- (30), intermediate- (20 and 15) and low-salinity (10) groupings (Figure 3). There was generally weak population-based clustering. The exception was the Blackwater Sound population which exhibited highly-similar reflectance spectra across all salinity treatments, except 10 (Figures 2 and 3).

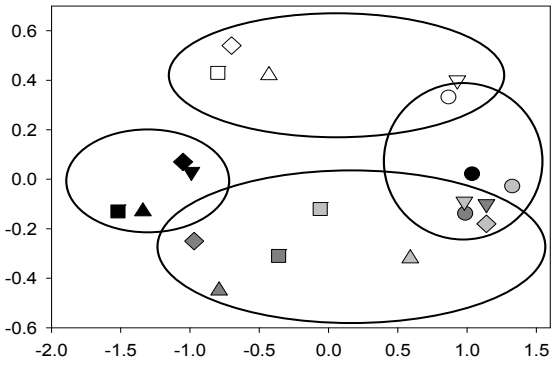

Figure 3. Two-dimensional MDS ordination of reflectance spectra similarity matrices among five populations (●=Blk, ■=Duc, ▲=Eag, ♦=Cal, ▼=Twn) of *S. radians* exposed to four salinity treatments (white=10, light grey=15, dark grey=30, black=30).

Discussion

The magnitude and spectral shape of coral spectral reflectance (*R*) are determined by absorption and fluorescence of several pigments localized in both the zooxanthellae and the host tissues (Hochberg et al. 2004). Healthy corals are optically dark and generally exhibit low *R* between 400-500 nm, increasing from 500-650 nm with a narrow chlorophyll absorption feature near 675 nm, then rapidly increasing at wavelengths greater than 680 nm (Holden and LeDrew 1998; Hochberg et al. 2004). Changes in environmental factors, such as temperature or salinity, may cause decreases in various pigment concentrations or expulsion of zooxanthellae from the coral host leading to spectral shifts and an overall increase in *R*. Thus, spectral distinctions between

healthy and non-healthy corals can be based on changes in the shape and magnitude of reflectance (Holden and LeDrew 1998, 2001).

Changes in *R* of *Siderastrea radians* from Florida Bay in response to experimental step-wise decreases in salinity indicate a high tolerance to hyposalinity stress, with a tolerance threshold between salinities of 10 and 15. Coles and Jokiel (1992) state that salinities below 15 sustained for more than two days will lead to coral mortality. That does not seem to be the case here as our measurements were obtained after four days at salinity 10 and six days at salinity of 15. Other coral species from coastal environments with fluctuating salinities, similar to Florida Bay, have also demonstrated tolerance to reduced salinities (Marcus and Thornhaug 1981, Muthiga and Szmant 1987, Manzello and Lirman 2003). *Siderastrea radians* from Blackwater Sound exhibited almost no change in *R* until salinity of 10 was reached and mainly below 500nm. *R* was elevated across PAR in the salinity 15 treatment for the two most interior basins (Eag and Cal). Blackwater Sound is a diverse basin with widely fluctuating salinities due to the influences of freshwater inflow from the Everglades via the C-111 canal and oceanic influences due to several cuts connected to the Atlantic Ocean (Hackney and Durako 2004). The small increases in *R* with decreasing salinities in the other four populations do suggest some loss of zooxanthellae pigments, but no bleaching of the colonies was observed. In addition, chlorophyll fluorescence measurements (F_v/F_m) indicated symbiont health was not acutely affected by decreasing salinities until a threshold was reached between salinities of 10 and 12, a salinity drop of roughly 20 from ambient (Chartrand 2008).

Different populations of *S. radians* across Florida Bay exhibited differing modes of *R*. At high salinity, three populations exhibited the brown mode *R* and the two most interior populations exhibited the blue mode *R*. Zooxanthellae pigments are responsible for the 575 nm peak characteristic of brown mode *R* (Hochberg et al. 2003). Coral-host pigments are thought to have strong absorbance in the 560-570 mn region leading to a loss of the 575 nm peak and production of a plateau-like shape from 600-650 nm that is characteristic of the blue mode *R*. Many coral taxa exhibit both blue- and brown-mode *R* that depend on the concentration of the coral-host pigmentation relative to that of the zooxanthellae (Hochberg et al. 2004). The shifts from "blue" or "brown" spectral shapes of *R* with decreasing salinities in two of the more estuarine populations that we sampled may indicate differing among-population salinity tolerances between the zooxanthellae and the coral host for *S. radians* occurring in different basins across Florida Bay. Thus, while coral *R* may be independent of taxonomic or geographic differences, reduced salinity does affect this parameter in *S. radians* from this marginal coral habitat and thus may provide a non-invasive metric for the health of this species within Florida Bay.

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Single-beam acoustic remote sensing for coral reef mapping

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Abstract. Several commercial single-beam acoustic seabed mapping systems have come to market in the last decade, but the potential for mapping coral reef habitats with these systems has not been systematically documented. Three datasets from the Florida Keys, USA, and the Bahamas, all acquired by a Quester Tangent Series V (QTCV), revealed that rock and sediment were reliably distinguished across multiple survey sites with accuracy ranging from 74%-86%. The utility of a simple rock/sediment classification scheme for assessing fish habitat and for objectively mapping habitat based on patchiness and relief metrics are discussed.

Key words: Acoustic seabed classification, QTC, fish habitat.

Introduction

Coral ecosystems that cannot be mapped with aerial or satellite imagery are both extensive and ecologically important. For example, over 55% of the Florida Keys National Marine Sanctuary (about 1540 square nautical miles) has not been mapped due to water depth or clarity limitations (FMRI 1998).

Acoustic systems are the natural solution for mapping areas where the seabed is not visible from overhead imagery. Single-beam, multibeam, or sidescan systems each may be used for acoustic seabed classification (Michaels 2007). The advantages of single-beam systems include relatively low costs, low data volumes, and easy portability.

Several studies have used commercial single-beam systems to map coral reefs (Hamilton et al. 1999; White et al. 2003; Moyer et al. 2005; Riegl and Purkis 2005; Riegl et al. 2007), but basic questions about what substrates can be reliably distinguished and how consistent classification schemes are in different areas have not been systematically explored. This project used a Quester Tangent Corporation (QTC) system to begin answering such questions.

Most evaluations of remote sensing technology or processing algorithms use data from a single study site. In contrast, the approach taken here was to compare results across multiple study sites in order to assess what seabed types could be mapped reliably by QTC across a variety of coral reef environments.

The objective was to determine what acoustic seabed classes were consistently distinguished across survey areas. The strategy used was to survey multiple sites using the same equipment, cluster the acoustic data from each site independently, and compare the accuracy of the resulting classifications.

Methods

The 50 kHz QTC Series V (QTCV) system described by Gleason et al. (2006) was used to acquire acoustic data during three surveys.

The first survey was conducted in the vicinity of Lee Stocking Island (LSI), Bahamas, on 16-20 June 2001 (Fig. 1). Approximately 145 km of track lines were surveyed along the bank top in water depths ranging from less than one meter to just over 10 m.

The second survey was conducted on 14 and 28 March, and 4 April 2002 offshore of Carysfort Reef, in the Florida Keys (Fig. 1). Fifty-two parallel transects, with a combined length of 124 km, were run across the upper shelf at depths of 3-35 m. This survey was described by Gleason et al. (2006).

The third survey was conducted at Fowey Rocks, also in the Florida Keys, about 45 km north-northeast of Carysfort (Fig. 1). The Fowey survey was conducted 12 and 20 October 2003 and included forty-one parallel transects, with a combined length of 72 km, across the upper shelf at depths of 3-40 m.

Acoustic data were processed with the IMPACT software package (version 3.4, QTC, Sidney, BC, Canada, 2004). Details of the IMPACT processing have been previously published (e.g. Preston et al. 2004; Gleason et al. 2006; Freitas et al. 2008). For the purposes of this paper, the key point regarding IMPACT is that the input to the program consisted of the raw echoes recorded by the QTCV and the output from the program consisted of: a) the optimum number of clusters into which the echoes should be split (as defined by Preston et al. 2004), and b) a label for each echo assigning it to one of the defined clusters.

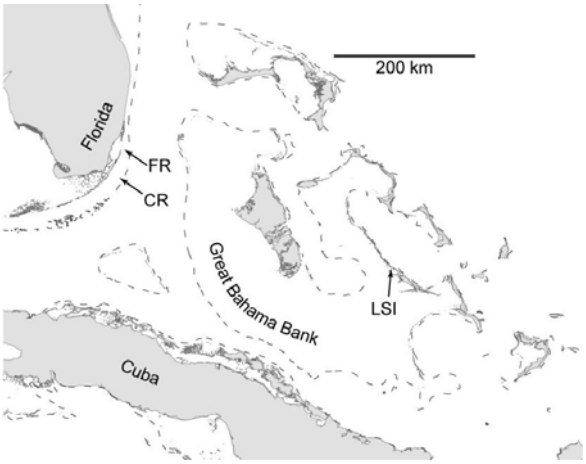

Figure 1: Location of Lee Stocking Island (LSI), Carysfort Reef (CR) and Fowey Rocks (FR) sites. Dashed line is the 200m isobath.

IMPACT's auto-clustering routine divides a dataset into distinct clusters based on echo shape, but, like any unsupervised classification routine, it cannot give these clusters descriptive names (e.g. reef, rubble, seagrass etc..). The clusters output from IMPACT, therefore, must be labeled by reference to other data sources. Cluster labeling for these surveys employed: comparison to satellite imagery, notes taken while snorkeling or drift diving, examination of the mean echo shapes for each cluster, bathymetric cross sections, and reference to previous seabed classifications at these sites (Gonzalez and Eberli 1997; FMRI 1998; Lidz et al. 2003; Louchard et al. 2003; Mobley et al. 2004).

Once the acoustic clusters had been labeled, they were quantitatively compared with "ground truth". The ground truth datasets acquired at each site were independent of the qualitative observations used for cluster labeling. Two types of data were collected for assessing the accuracy of the acoustic classification. Downward looking video images were acquired during the LSI survey, which was shallow enough and in clear enough water that the seabed was always visible from the surface. In contrast, the seabed was not visible from the surface at all times during the Carysfort and Fowey rocks surveys, due to deeper and less clear water. Therefore, at Carysfort and Fowey diver-based observations were acquired.

The video from the LSI survey was acquired with a Sony TRV 900 camera in an underwater housing that was mounted to the same pole that supported the transducer used for the survey (Fig. 2). Video was acquired in time-lapse mode, so that an entire day's worth of surveying could fit on one video tape. In time-lapse mode, the camera was set to acquire video (at full frame rate) for two seconds and then to pause for 28 seconds.

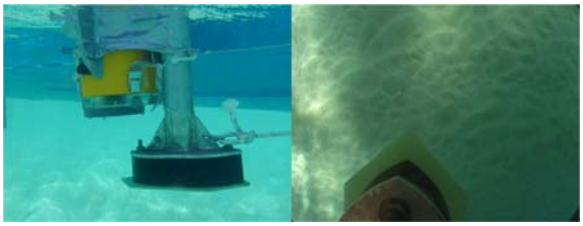
Figure 2: Left: Underwater photograph of pole-mounted transducer and video camera housing, as used for the LSI survey. Right: Sample frame grabbed from the video over a sandy seabed.

The entire LSI video data set consisted of 1502 two-second long clips. A single frame was extracted from each clip for analysis. For each frame, an analyst estimated the percent of rocky and sandy substrate. At the beginning of the day, the camera's clock was synchronized with a GPS unit. Using the time code embedded with each frame, the locations of the frames were determined from daily GPS tracks.

The diver-based observations collected at Carysfort Reef (N=22) and Fowey Rocks (N=15) followed the Bohnsack and Bannerot (1986) stationary reef visual census (RVC) method. The RVC protocol focuses on the collection of fish population data, but habitat data, including estimates of the percent of the seabed covered by rock, rubble, and sediment, are also collected (McClellan and Miller 2003). Estimates of substrate are the portion of the RVC dataset used for this analysis.

An error matrix (Congalton and Green 1999) was constructed for each survey site for comparison of the acoustic classification with the video/diver estimates of substrate. The comparison was made between each ground truth sample and the closest acoustic echo to that point. One refinement of the standard error matrix technique was necessary. The video/diver data was expressed as a fraction; the substrate at each point was X% sediment, Y% hard bottom, and Z% rubble. The acoustic classes, on the other hand, were discrete, so each entry in the error matrix was divided proportionally by the video/diver-estimated substrate (Gleason et al. 2006 have a sample calculation.)

Results

The LSI data clustered into nine acoustic classes. Only four of these classes, however, made up 96% of the total number of echoes. The cluster labeling process indicated that one of the acoustic classes, with 26% of the echoes, corresponded to hard bottom while the other three classes, with combined 71% of the echoes, corresponded to sediment. Comparison with the LSI video dataset indicated that the hard bottom/sediment acoustic classification had an overall accuracy of 74% (Fig. 3).

The Carysfort data clustered into seven acoustic classes. Only three of these classes, however, made

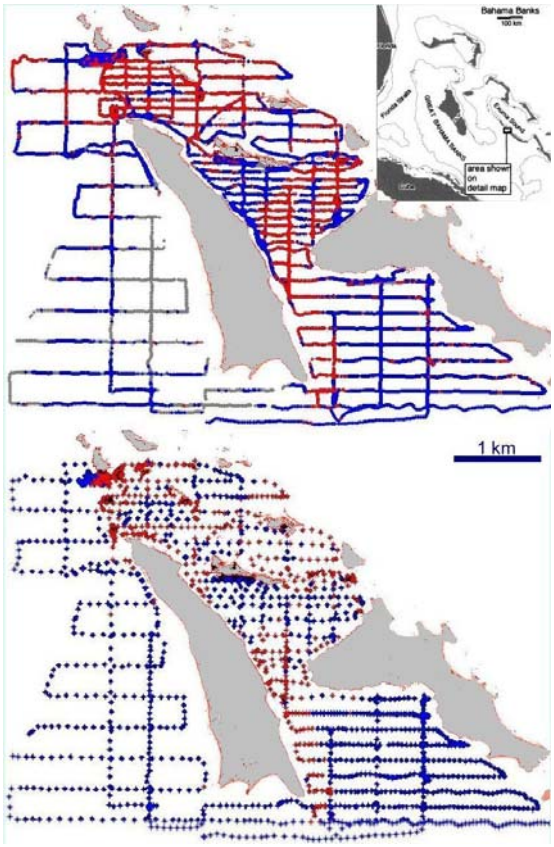

Figure 3: LSI acoustic classification (top) and video classification (bottom). Red and blue points in both plots indicate hard bottom and sediment, respectively. The two additional sediment classes in the acoustic classification are shown in grey (top). Islands are shown in grey in both plots.

up 94% of the total number of echoes. The cluster labeling process indicated that one of the acoustic classes, with 46% of the echoes, corresponded to hard bottom while the other two classes, with combined 48% of the echoes, corresponded to sediment. Comparison with the RVC substrate dataset indicated that the Carysfort hard bottom/sediment acoustic classification had an overall accuracy of 86%.

The Fowey Rocks data clustered into six acoustic classes. Only four of these classes, however, made up 97% of the total number of echoes. The cluster labeling process indicated that two of the acoustic classes, with combined 63% of the echoes, corresponded to hard bottom while the other two classes, with combined 34% of the echoes, corresponded to sediment. Comparison with the RVC substrate dataset indicated that the Fowey Rocks hard bottom/sediment acoustic classification had an overall accuracy of 78% (Fig. 4).

Discussion

The results showed that rock was well discriminated from sediment using the QTCV. Overall accuracy for the rock/sediment classification in the three surveys ranged from 74% to 86%. Many maps with a coarse level of descriptive resolution that were derived from satellite imagery have overall accuracy in this same range (Mumby et al. 1997; Andrefouet et al. 2003).

It is worth considering whether a rock/sediment classification scheme is too simple to be useful. Even though the overall accuracy of the simple two-class acoustic seabed maps was comparable to coarse-level maps derived from satellite imagery, most image-derived seabed maps have more than just two classes. One difference between satellite or aerial imagery and acoustic data is that bathymetry is inherently part of the acoustic data collection, and is therefore available to complement the seabed classification. Adding even a simple rock/sediment classification to traditional bathymetric data has a strong potential to benefit habitat mapping.

One example of the benefits of adding a simple rock/sediment classification to traditional bathymetric data for habitat mapping is the ability to discriminate outcropping parts of the seabed. Hard bottom is known to be important habitat for many types of fish. Sometimes substrate can be inferred from topographic profiles, but in other cases interpreting the bathymetry alone can be misleading. Figure 5 shows an example from the Florida Keys where bathymetry alone provided a misleading picture of habitat.

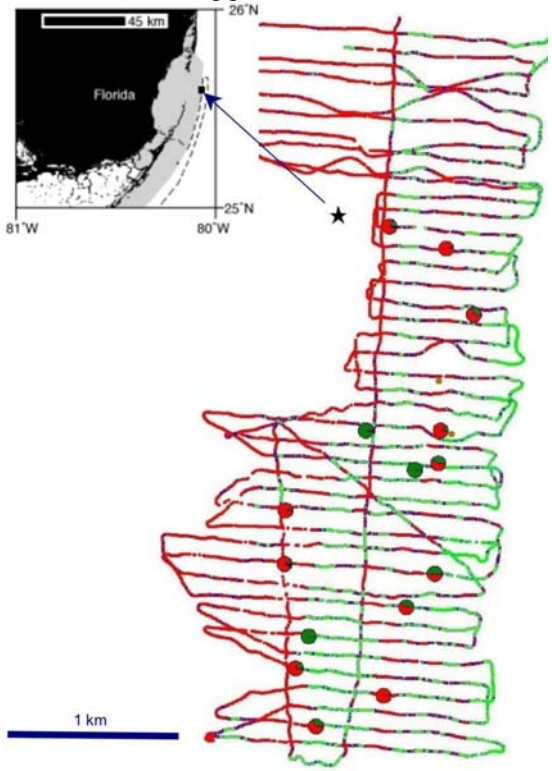

Figure 4: Fowey rocks acoustic classes (track lines) and diver estimated substrate (pie charts). Red and green represent hard bottom and sediment substrate for both types of symbols.

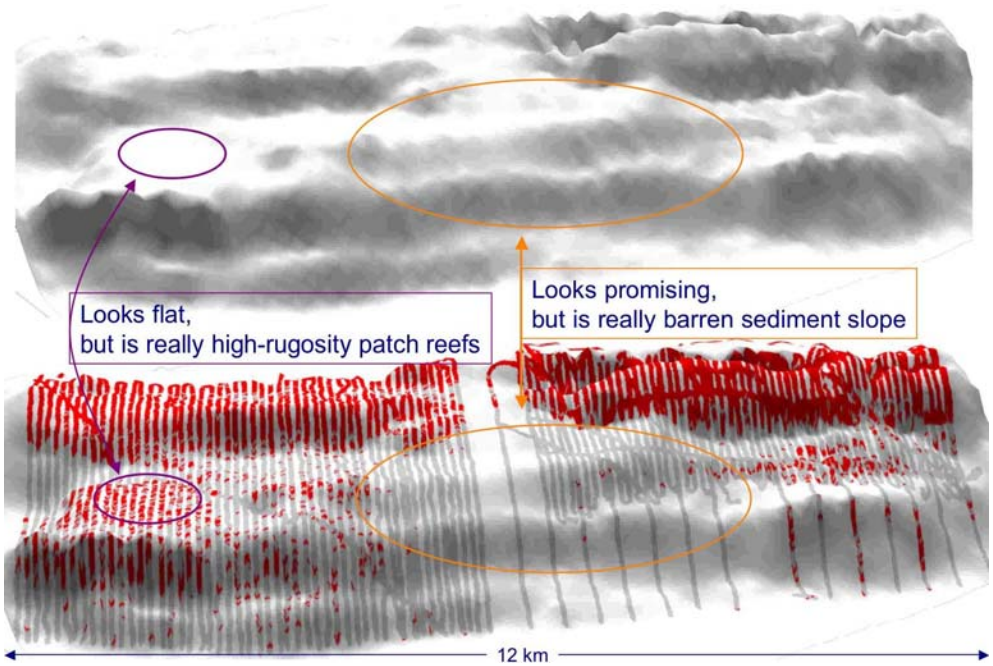

Figure 5: Example of the utility of rock/sediment seabed classification in interpreting bathymetry for fish habitat. Top panel: sunshaded bathymetry from single-beam echosounder depths interpolated to a 30 m grid along the upper slope seaward of Carysfort and Watson's Reefs, Florida Keys, USA. Bottom panel: Survey track lines superimposed on the same sunshaded bathymetry as the top panel. The track lines are colored red for hard bottom (rocky) and gray for sediment substrate. In both panels, oblique view is from the ESE, total along shelf distance is about 12 km, and depth ranges from 3 to 60 m.

Based on bathymetry alone (Fig. 5, top), the area in orange appears to be promising fish habitat because it contains two parallel ridges with steep slopes, and the area circled in purple appears to be a flat, featureless plain. When considering seabed type in addition to bathymetry (Fig. 5, bottom), a different interpretation becomes apparent; sediment covered the area in orange, providing little shelter for fish, while the area in purple was covered with small patch reefs, which would generally provide excellent reef fish habitat.

A second benefit to habitat mapping resulting from the addition of a simple rock/sediment classification to bathymetry is the potential to create an objective habitat classification scheme based on patchiness and relief. Franklin et al. (2003) proposed such a habitat classification scheme for coral reef environments. Patchiness was defined as the percent of the seabed within a certain radius of the point being classified that was covered with sediment. Relief was defined as the depth range within a certain radius of the point being classified.

Miller et al. (2008) used QTCV data and the Franklin et al. (2003) classification scheme to produce a benthic habitat map for the Navassa National Wildlife refuge. One of more than 100 transects used to create the Miller et al. (2008) map is shown in Figure 6; it illustrates the potential of QTCV data as input to the Franklin et al. (2003) classification scheme. The top portion of Figure 6 shows the transect plotted with echoes colored by substrate, while the bottom shows the transect plotted with echoes colored by both substrate and relief.

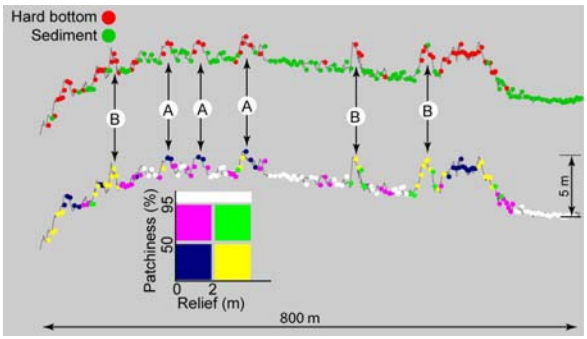

Figure 6: Cross shelf profile of part of the Navassa Island insular shelf. Top: Echoes colored by substrate only, red for hard bottom and green for sediment. Bottom: The same transect with echoes colored by a combination of local patchiness and relief. Patch reefs marked A and B were low and high relief, respectively.

Two types of patch reefs are highlighted along the transect (Fig. 6). Reefs labeled "A" and "B" had similar cross-shelf width, but those labeled "B" had higher relief. Considering substrate only, these reefs were all the same class (hard bottom), but considering both patchiness and relief, the low and high relief patch reef categories were objectively discriminated (yellow vs. blue class).

Franklin et al. (2003) produced a habitat map of the Dry Tortugas using a classification scheme based on patchiness and relief, but the map was based on

qualitative interpretation. The advantage of using a single-beam acoustic approach, as described above, for this type of classification is that it is objective and automated.

Conclusions

The results of this study showed that the QTCV commercial single-beam acoustic seabed classification system discriminated rocky from sediment substrate with about 80% accuracy. This result was confirmed with surveys at multiple sites using the same QTCV system and different methods for ground truth.

Two examples were given demonstrating that even a simple rock/sediment classification can improve habitat mapping of coral reefs. One example showed that using substrate information improved an interpretation of fish habitat based on bathymetry alone. The second example showed that QTCV single-beam data were capable of objectively classifying habitat based on patchiness and relief, whereas previous efforts to do so had relied on subjective analyst interpretation.

Due to the robust capability to extract bathymetry and basic rock/sediment substrate classification, combined with low cost and portability, single-beam systems have the potential to complement other survey methods in coral reef mapping efforts.

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NASA airborne AVIRIS and DCS remote sensing of coral reefs

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Abstract. To adequately image through a water column and delineate variation in coral reef ecosystem benthic cover types, sensors having high spatial resolution, high spectral resolution and high signal-to-noise are needed. Further, there is a need to better understand the optical properties of coral reefs, seagrass, other benthic cover types, and water column constituents from field-collected data so current and future remote sensing can be optimized for coastal zone ecosystem research and management. In August 2004, we flew the Airborne Visible Infrared Imaging Spectrometer (AVIRIS) and Cirrus digital camera system (DCS) on a NASA ER-2 over Puerto Rico. Also, in December 2005, we flew AVIRIS on a Twin Otter over priority sites for Puerto Rico for assessment of the 2005 Caribbean coral reef bleaching event. For each of these deployments, we collected coincident spectral data from dominant bottom types and coral under various health conditions using a hand-held spectroradiometer. These spectral data are being used to classify different benthic cover types present within the AVIRIS imagery. An overview of the airborne missions and coincident field data collection for calibration and validation of the airborne remote sensing data are presented along with preliminary image and field-collected spectral data products.

Key words: Coral reefs, hyperspectral, AVIRIS, airborne remote sensing.

Introduction

The photosynthetic pigments in the symbiotic algae (zooxanthellae) of corals and the general establishment of corals in shallow well-lit waters enables the detection of spectral information from corals through a clear shallow water column with a remote sensing instrument. Corals display distinct reflectance features between 550 and 650 nm related to the densities of chlorophyll-*a* and accessory pigments in their tissue (Holdren and LeDrew 1998; Myers et al. 1999; Hochberg and Atkinson 2000; Hochberg et al. 2003). Research has shown that spectral distinction of reef bottom types (i.e., coral, algae, and carbonate sand) is possible using field spectroscopy (Clark et al. 2000; Hochberg and Atkinson 2000; Andrefouet et al. 2001; Lubin et al. 2001; Hochberg et al. 2003; Wettle et al. 2003). Of further interest is the identification of spectral features indicative of degradation in reefs which could lead to better ecological assessment (e.g., relative health and biodiversity) and forecasting (Call et al. 2003; Hochberg et al. 2003).

Because only the visible range of the electromagnetic spectrum can penetrate deep enough into the water column to reach shallow-water benthic cover types, there is a unique requirement for not only high spatial- but high spectral-resolution remote sensing data to adequately discriminate benthic cover types, variations due to disturbance, and changes in reef ecosystems in an optically complex environment. Recently, remote sensing of coral reef communities has evolved from purely multispectral (e.g., Landsat) to improved mapping using higher spatial resolution multispectral (e.g., Ikonos) (Mumby and Edwards 2002; Purkis, 2005) to advanced hyperspectral (e.g., AVIRIS) techniques (Goodman and Ustin 2007).

Hyperspectral sensors provide a greater range of fidelity when discriminating between bottom types, because details of spectral shape and pattern are better revealed using numerous narrow bands rather than fewer broad multispectral bands (Holden and LeDrew 1999; Zimmerman and Wittlinger 2000; Butler and Hopkins 1970). As the water depth increases, light in longer wavelengths (>600 nm) is attenuated more readily than in green to blue wavelengths (shorter wavelengths), leaving only blue and green wavelengths (400-600 nm) with which to differentiate corals and other substrates (Green et al. 2000; Holden and LeDrew 2002). We are leveraging the high number of channels and narrow bandwidths of NASA's airborne hyperspectral remote sensor, AVIRIS, to provide a more comprehensive

assessment and mapping of shallow coastal resources (Guild et al. 2007).

The objectives of the coincident AVIRIS and field data collection missions included 1) mapping coral reef benthic type and degradation and 2) interpretation of reef biodiversity and variability.

Materials and Methods

Study Site. Our field site is the La Parguera shelf, southwestern Puerto Rico (17º 57' N, 67º 02' W: Fig. 1). La Parguera has numerous bank reefs that protect the shore from intense wave action, resulting in extensive seagrass meadows and a coastline dominated by mangroves with algal plains, sandy lagoons and two bioluminescent bays. This area has minimal input of suspended sediments or dissolved organic matter from land sources due to low precipitation and absence of riverine input. Annual precipitation ranges from 500 to 1,200 mm with a dry period from December through April.

Figure 1: The study site, designated by a star, is La Parguera in Southwestern Puerto Rico. This AVIRIS image was acquired during the 2004 AVIRIS mission. This RGB composite image displays channels 31, 21, and 10 at wavelengths of 655, 559, and 453 nm, respectively.

Airborne Missions. On August 19, 2004, AVIRIS and DCS were flown along the majority of coastal Puerto Rico, including Vieques Island to the east. The altitude of the NASA ER-2 was approximately 20 km, resulting in 17 m AVIRIS pixel resolution and 7 m DCS pixel resolution.

In mid-December 2005, in response to the most devastating regional-scale coral bleaching event on record in the Caribbean (Wilkinson and Souter 2008), AVIRIS/DCS was again flown over sites in Puerto Rico, as well as the US Virgin Islands, to investigate areas of coral bleaching. AVIRIS and DCS were flown onboard the NASA Twin Otter platform at an altitude of approximately 3.5 km for sensor spatial resolutions of 3.5 m and 0.7 m, respectively. Further, an additional DCS camera was flown on a low-altitude aircraft the week following the AVIRIS mission in Puerto Rico acquiring 15 and 30 cm spatial resolution data at 1 and 2 km altitudes, respectively.

AVIRIS has 224 contiguous spectral channels with wavelengths from 380 to 2500 nm and a 10 nm nominal bandwidth. AVIRIS has a high signal-to-noise ratio and has 32 channels in the visible wavelength range (400-700 nm), which are valuable for use in shallow water environments. The signal-to-noise ratio varies by wavelength from about 1000 in the visible region to about 500 in the infrared region and also exhibits reduced signal-to-noise in atmospheric absorption wells (e.g., water vapor) in bands located at or very close to wavelengths where atmospheric absorption appears (e.g., reduced reflectance in AVIRIS bands 62-64 corresponding to 929.0, 945.1, and 954.5 nm due to water vapor absorption around 945 nm).

The Cirrus DCS is a high resolution, medium format, color-infrared digital camera. The camera uses a Zeiss lens and provides 16-megapixel resolution. The camera can operate in visible (natural color) or color infrared mode. Visible mode (e.g., red, green, blue channels only) was used for our deployments.

In Situ Measurements. Underwater field sampling of four patch reefs across La Parguera shelf and surface measurements were conducted during both airborne deployments to support the classification of AVIRIS imagery based on benthic type, and for validation of AVIRIS atmospheric and sunglint correction schemes. A summary of the measurements are as follows: *in situ* reflectance (R) of corals and other benthic communities, spectral water attenuation coefficients (K_d), chlorophyll, turbidity, surface remote sensing reflectance (R_{rs}) for calibration, and sunphotometer measurements of aerosol optical thickness (AOT) for atmospheric correction.

For water column characterization and correction of reflectance data, we use Hydrolight (Sequoia Scientific) radiative transfer algorithms. Water column light attenuation coefficients (K_d) were calculated from the average of three to five spectra of a spectralon panel at three depths using a GER 1500 (Spectra Vista Corp.) handheld spectroradiometer in an underwater housing. These K_d estimates serve as an independent verification of the range of appropriate K_d values used for Hydrolight. Only two depths are necessary for the calculation, but extra K_ds were collected to avoid sensor saturated data from wave focused light effects on the spectralon.

Spectra from bright and dark validation targets (10 m x 10 m) were measured with the GER 1500 during the 2005 overflights. Aerosol optical depths (AOT) were measured using two Microtops sunphotometers (Solar Light Co., Inc.) with calibrated filters for aerosols and ozone, both operated onboard

boats at the patch reef study sites during the 2004 and 2005 overflights. These AOT estimates provide independent estimates for evaluating the range of appropriate AOT values used for the atmospheric correction algorithm (Lobitz et al. 2009; these proceedings).

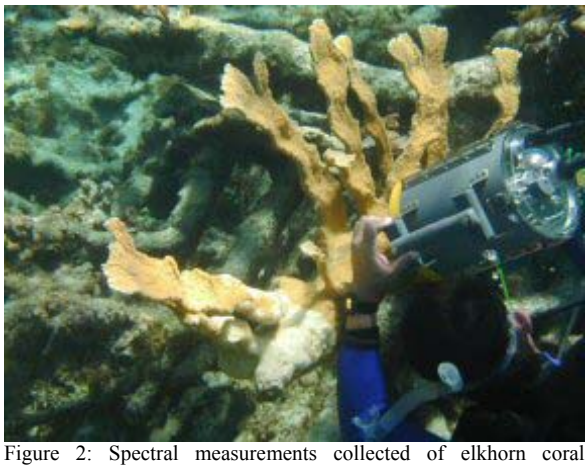

Figure 2: Spectral measurements collected of elkhorn coral (*Acropora palmata*) using the GER 1500 spectroradiometer in underwater housing.

Spectral measurements of dominant benthic cover types (including ecological variation) along 10 m transects were collected at each reef site for calculation of reflectance of benthic types and for spectral library input into classification algorithms for delineation of benthic cover types and for evaluation of variability within and between benthic cover types. The distribution of these transects were randomly placed and stratified to represent example coral stands in forereef, reef crest, and back reef areas. The linear transects were positioned to sample an extent of a reef patch that was mostly homogeneous and would dominate even a 17 m AVIRIS pixel. Three to five spectra were taken each for the spectralon and benthic type (Fig. 2) at 1 m intervals on both sides of the metric tape along each 10 m transect. GPS positions were recorded at the transect endpoints and dGPS positions were established at a later date. Further, additional spectral library measurements and GPS positions of other dominant bottom types were taken around the transect tape to use in image classification. A photo record was taken of each GER spectral reading. The perimeters of several large coral stands were also recorded as polygons of GPS points to train/validate benthic classification.

Image Processing. We are employing new approaches for hyperspectral data analysis to study coral reef biology and optical properties and to evaluate the inherent spectral heterogeneity of cover types within pixels (Goodman 2004; Goodman and Ustin 2007; Roberts et al. 1998). *In situ* spectral libraries, collected specifically from sites in the study area, are being used in spectral mixture analysis algorithms for subpixel benthic classification and the assessment of changes in reef composition, particularly biodiversity.

Raw AVIRIS data are being processed utilizing a sequence of image processing steps to resolve the complex interaction of atmospheric conditions, bathymetry, sea surface state, water optical properties and bottom composition (Fig. 3).

The analysis starts with three phases of image preprocessing, which includes stray light suppression, atmospheric correction and sun glint removal, and then image processing utilizes a semi-analytical optimization model to retrieve bathymetry and water properties throughout the study area. Using field spectra data representing the dominant benthic components (e.g., spectral endmembers for sand, coral, and algae), a constrained non-linear unmixing model is utilized to classify the benthic substrate as a function of the fractional contribution from each endmember. The final step utilizes field observations to assess the accuracy of the resulting image products.

Preprocessing. The first step of the image preprocessing is suppression of the near-infrared glow (i.e., anomalously large values) in low-light AVIRIS 2004 and 2005 imagery. This glow was caused by stray-light leakage following an upgrade to the instrument prior to the 2004 flight season. It is suppressed by calculating a correction based on the glow's cross track profile and the difference between the central stripe of "good" data and the adjacent incorrect pixel values that include the contribution from the stray-light. Details of the stray light suppression can be found in Lobitz et al. (2009; these proceedings).

The second preprocessing step is atmospheric correction, performed using Tafkaa, an algorithm for atmospheric correction of imaging spectrometry data under development at the Naval Research Laboratory designed to address the confounding variables associated with shallow aquatic applications (Gao et al. 2000; Montes et al. 2003; Montes et al. 2001). The Tafkaa algorithm includes atmospheric gaseous absorption and aerosol corrections as well as pixel location-specific solar and viewing geometry to retrieve per-pixel water surface reflectance. Details of the atmospheric correction methods can be found in Lobitz et al. (2009; these proceedings).

The third preprocessing step, a spectral normalizing procedure based on Hedley et al.'s (2005) variation of Hochberg et al.'s (2003) method was used to reduce the effects of sun glint (i.e., specular reflection from the water surface). In this method the slope of the regression line between pixel values from a NIR-band (750 nm) and each of the visible bands is

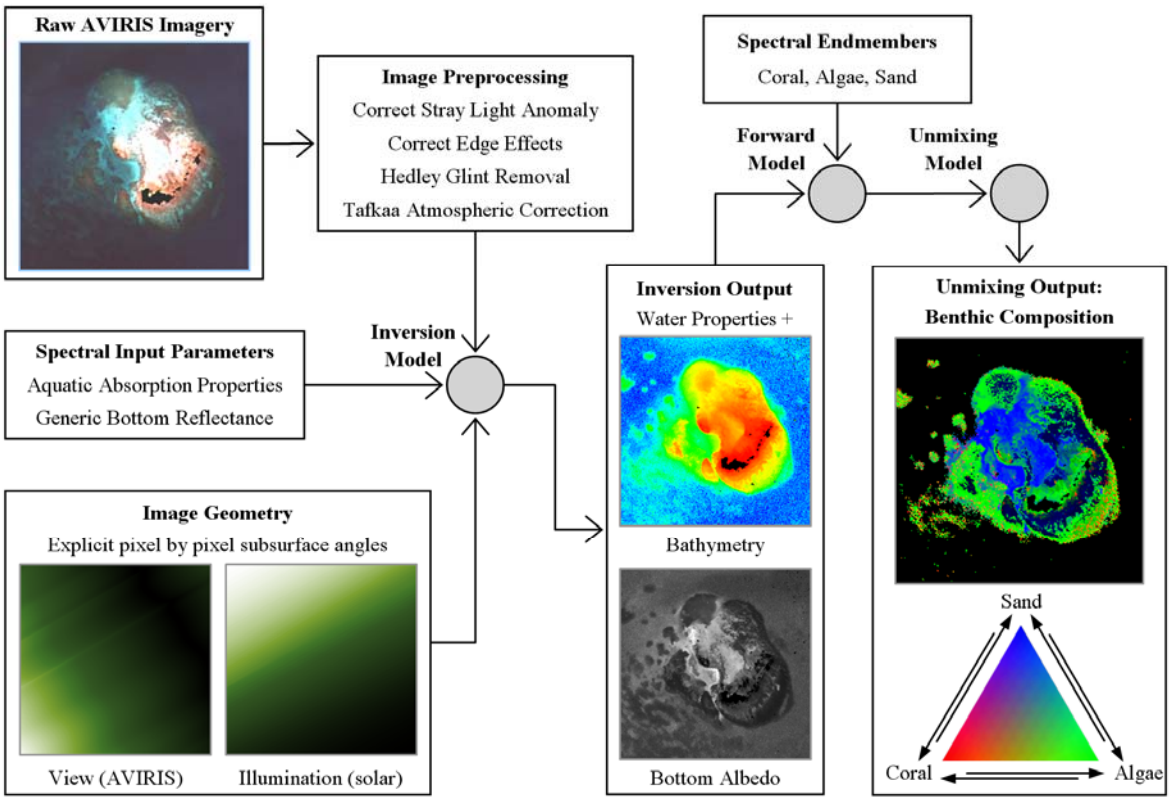

Figure 3: Overview of AVIRIS processing steps. On the left side are the inversion model processing inputs (corrected AVIRIS, spectral input parameters, and image geometry) and outputs (water properties, bathymetry, and bottom albedo) using a subset of the AVIRIS data for San Cristobal patch reef. On the right side are the forward model inputs (spectral endmembers, inversion output, spectral input parameters, and image geometry), integrated with the preprocessed AVIRIS imagery in the unmixing model to produce the benthic composition (sand, coral, and algae)

computed over a sample containing sun glint. This slope is then used to reduce the values in each visible band, relative to the difference between the NIR-band minimum value within the training area and the location-specific NIR-band value.

Inversion Model and Unmixing. Following image preprocessing corrections, a semi-analytical inversion model is used to retrieve estimates of bathymetry and water properties from measured surface remote sensing reflectance to correct for water column effects in the imagery (Fig. 3). Aquatic absorption properties are a combination of absorption properties of pure water and empirical spectra derived from field data and Hydrolight runs (Lee et al. 1998; 1999). The generic bottom reflectance used in the model is an average sand spectrum from the study area. Image geometry data indicate calculated variations in view and illumination angles within the AVIRIS image (Fig. 3). The Inversion Model is applied to derive water properties, bathymetry, and bottom albedo.

We then proceeded with defining spectral endmembers from measured field data and performed the benthic classification using unmixing techniques (Fig. 3) (Goodman and Ustin 2007; Goodman 2004).

Generic spectral endmembers of coral, sand, and algae (including a shade endmember) are used together with the inversion model outputs (water properties, bathymetry, bottom albedo) as well as the spectral input parameters and image geometry in the Forward Model. The next step is to take this information and run the Unmixing Model on the AVIRIS data to output a benthic composition image of coral, sand, and algae.

Results

The output benthic composition shows a predominance of algae and sand. The prevalence of algae in the reef areas is reasonable due to the pervasiveness of reef stands of rubble overgrown with algae. Since we have not delineated spectra for seagrass, seagrass in the back reef zone is classified as algae. We are currently evaluating the preliminary benthic composition image product and rerunning the unmixing model with additional spectral endmembers (including seagrass) to evaluate variation in the benthic composition output image (Fig. 3). The next step is to utilize field observations and DCS imagery to assess the accuracy of the resulting image products.

Discussion

Our research strategy includes refining the classification and evaluating our spectral transects with multiple endmember spectral unmixing as an additional approach to benthic classification.

A rich data set from the AVIRIS airborne and field deployments provide a unique opportunity to advance studies in changes in coral reef ecological structure and biodiversity. The magnitude of field studies ongoing in our study sites, as well as data collected specifically to support the airborne deployments, provide a strong baseline of information for thorough analysis of the reef ecology and biodiversity in these regions. The lessons learned about which spatial resolutions are most appropriate for sensing coral reef benthic communities will provide specification requirements for future hyperspectral sensors onboard conventional aircraft, unmanned aircraft systems (UASs), and spaceborne platforms.

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An investigation of seagrass patterns at Alphonse Atoll, Seychelles: Linking structure to function in marine landscapes

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ABSTRACT: The idea of landscapes as shifting patch mosaics, structured by a range of biological and physical stochastic forces, is well suited to shallow tropical environments, where seagrass patches lie within a matrix of soft sediments or rocky substrates. The interaction of wave fields and tidal currents with carbonate sediment transport can result in linear morphologies of reef flat material with alternating sand tongues and seagrass beds. Patch-level metrics capture phenomena such as linearity in one variable, which can be evaluated over a gradient of predictable environmental change. Interrogating the statistical properties of patch ensembles enables the links between observed structures and the processes that govern them to be empirically investigated. This study demonstrates how habitat maps derived from remotely sensed Compact Airborne Spectrographic Imager (CASI) data can be used to investigate critical controls of landscape mosaics through the application of geostatistical techniques to Alphonse Atoll, Seychelles.

Key words: Landscape ecology, patch linearity, remote sensing

Landscape ecology is characterised by the empirical investigation of the structure and function of land surface features (Forman and Godron 1986), with particular regard to spatial heterogeneity on biotic and abiotic processes (Risser et al. 1984). The broad scale of a landscape implies that internal processes can be observed across a spectrum of spatial scales, by dividing landscapes into patches, providing a tool for the representation of reality that can be readily understood. Patch-level metrics capture a feature of interest in one variable, which can then be evaluated over a gradient of predictable environmental change. In this way, ecologists are able to quantify and empirically link structure and function at the landscape scale.

Four key requirements for seagrasses are a marine environment, adequate rooting substrate, sufficient immersion in seawater and illumination to maintain growth (Hemminga and Duarte 2000). Seagrass landscapes are composed of seagrasses (marine angiosperms) and unvegetated sediments. Studies of seagrass dynamics at the landscape scale have generally focused on mapping historical change in distribution and cover over time, doing little to link observed structures to underlying processes driving change. Across a gradient of increasing hydrodynamic activity, seagrass beds form patterns that range from continuous meadows to widely dispersed, discrete patches (Fonseca and Bell 1998).

Terrestrial landscape ecologists have developed paradigms on island biogeography and fragmentation that have not crossed over to marine landscapes, which have traditionally focused on patch dynamics (Fonseca and Bell 2006). Sub-littoral landscapes are often viewed as a shifting biological mosaic of multistate systems structured by competition, grazing and predation. Such a viewpoint argues for a shift in focus, from the individual patch to the statistical properties of the entire ensemble. Landscape ecology techniques have emerged as a useful means of investigating functional drivers, such as the influence of wave exposure on community composition (Turner et al. 1999) and the relative influence of internal and external drivers (Fonseca et al. 2008).

Location

Alphonse Atoll (Figure 1) lies at the southern end of the Amirante ridge on the southwestern margin of the Seychelles Plateau, western Indian Ocean. The atoll covers an area of approximately 6x4km and consists of a narrow fore-reef shelf, wide peripheral reef flats and a dish-like lagoon reaching depths of 10m (Spencer et al. 2000). Alphonse is subject to a semi-diurnal tidal regime of range ~2m, upon which reversing monsoons control the direction and strength

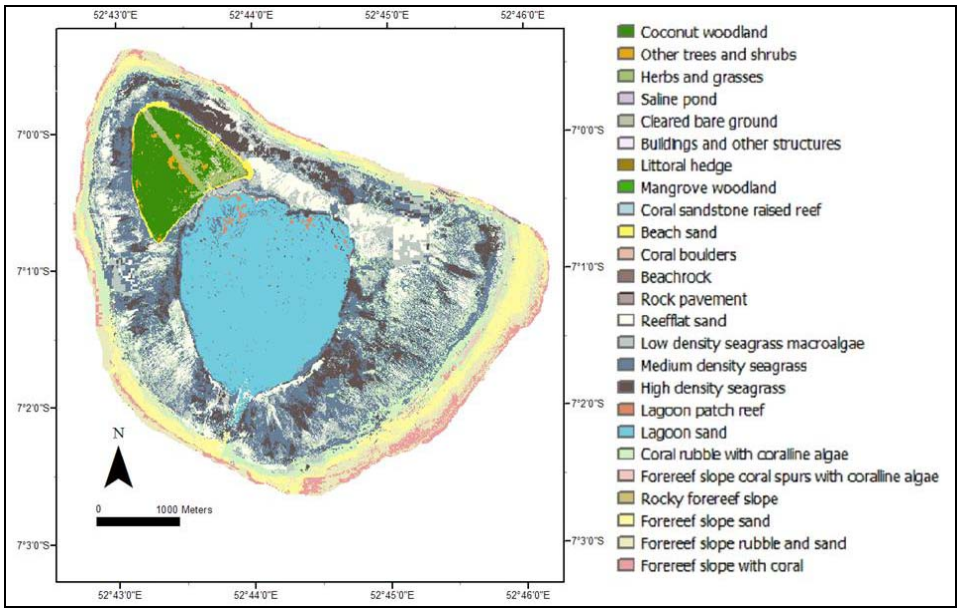

Figure 1. A habitat map of Alphonse Atoll, Southern Seychelles, West Indian Ocean (7°S, 52°E).

of surface currents. However, for the majority of the year, Southeast Trade winds dominate incident wave activity. The upper fore-reef exhibits distinct coral spur and groove formations. These extend around the atoll perimeter, being particularly well developed on the south-east and north-western windward coastlines. A distinctive feature of the adjacent reef flat is alternating linear tongues of seagrass on carbonate sediments. Similar features can be observed on many Indian Ocean reef flats and the aim of this paper is to investigate the functional role of spur and groove morphology, along with wave power, as drivers of these linear seagrass structures on the reef flat

Methods

A habitat map was generated from remotely sensed data acquired with an aircraft-mounted Compact Airborne Spectrographic Imager (CASI). Seventeen spectral bands of data were acquired at a spatial resolution of 1 sq.m, yielding synoptic coverage of the area of interest. Following geocorrection, flight strips were mosaiced using a band-wise linear colour balancing model to minimise across-track variance, with histogram matching to adjust for radiance offsets. Image bands were corrected for the effects of light absorption and scattering in the water by approximating the vertical radiative transfer through a water column to a logarithmic decrease with increasing depth (Lyzenga 1981). Training areas derived radiance measures over a number of spectral subclasses to build up statistical populations of habitat classes apparent in the image feature space. A maximum likelihood classification was performed on depth invariant bands, assigning each pixel of the image to the most likely habitat class on the basis of statistical probability. Map accuracy was assessed by exporting 283 polygon centroid coordinates to a hand-held GPS and comparing the corresponding location in the field to the habitat type recorded on the map. Overall accuracy was defined as the total number of correct patches divided by the total number of patches in the validation assessment.

Field data were collected on spur and groove depths and wave power was empirically modelled to derive information on potential drivers of seagrass patch structure as a response variable. Gustafson and Parker (1992) developed a metric for assessing patch linearity based on the premise that elongated patches of a given area are comprised of pixels closer to their edges than square patches of the same area:

$$LINEAR = \frac{\left[\frac{a_{ij}^{*}}{(2b-r)^2}\right] - 1}{a_{ij}^{*}} \qquad \textit{Equation 1.}$$

where a_{ij}^{*} =area of patch ij in terms of number of cells, and b = average cell value of the pixels comprising the patch. r = 0 if the patch contains side by-side pixel rows; 1 if not.

Six analysis windows were established in the centre of the reef flat to investigate seagrass community structure around the atoll. Linearity was calculated step-wise for each patch by calculating Euclidean distance from patch boundaries and averaging pixel values across patch spatial extents. A focal rank operator was used to record the number of pixels in

the immediate neighbourhood with a value less than the centre pixel. For each pixel of the input distance surface local maxima were returned as zero values in an output thematic layer. An optimal sample window size was defined using an experimental semivariogram. This was calculated by sequentially comparing the linearity of each individual patch to the rest of the patches in the map via the Moran statistic and plotting these values against the distance between patches. The lag distance at which the maximum semivariance was reached for linearity of all seagrass patches within the landscape was found to be 300m; hence this was adopted as the sample window dimensions.

Field measurements of groove depths were made using a Norcross DF2200PX handheld bathymetric sounder. A 50m transect was established along a defined bearing, along which a diver swam at a constant depth above the spur and groove morphology, perpendicular to the direction of groove alignment. GPS locations were recorded at both ends of the transect and depth measurements were recorded at 1m intervals.

Wave power was calculated using an amended version of the approach described by Roberts (1974). Transects of 5km length were established across the atoll cross section, running towards the lagoon centre, at ten degree intervals around the atoll. A bathymetric map facilitated derivation of a shoaling coefficient for each pixel for a given depth to wavelength ratio. Additionally, a refraction coefficient was calculated from the change in angles between the waves and shoreline and wave velocity (Weigel 1964). Mean wave height and period were hindcast for the outer point of each transect using data on wind strength, frequency and fetch taken from the Indian Ocean volume of the *Marine Climatic Atlas of the World* (US Navy 1958). Wave height was then computed for each pixel along the transect as a function of the wave height at the previous interval, change in shoaling coefficient, and change in refraction coefficient.

The influence of water movement was investigated on seagrass structure by carrying out regressions of patch linearity against independent variables at two spatial scales. In the first instance, descriptive bivariate regressions were used to explore the separate influence of "groove depth" and "wave power" on the linearity of patch assemblages within analysis windows. Geographical variation in the residuals of these bivariate models was reviewed to investigate additional model covariates. Multivariate regression was used inferentially to extend statements about the mean patch statistics from sample windows to the patches comprising the rest of the atoll habitat map. Tests were run to ensure the assumptions of multiple regression were met.

Results

Figure 2 summarises the geographical distribution of the information collected. Key points to note include:

-A good quality image classification was achieved, providing a clear and accurate representation of the heterogeneity apparent in the raw image (overall accuracy = 77%)

-Upper terrace spur and groove adjacent to the survey windows was well pronounced on the southeast and northwest faces of the atoll, where deep grooves were prevalent (Fig. 2).

-Lower relief spur and groove was recorded adjacent to the remaining sites, where wider, less frequent spurs divided shallower grooves.

-The wave power model indicated that the southeast-facing coastline of the atoll was subject to higher energy levels than the remainder of the atoll

-Computed values for the linearity metric (Equation 1) ranged between 0.22 and 0.67 for seagrass patches on the reef flat.

Bivariate regressions of groove depth and wave power with linearity values for seagrass patches falling inside adjacent survey sites on the reef flat revealed moderate positive correlations (Table 1). This suggested that the better "developed" the spur and groove morphology, the more linear the adjacent seagrass patches.

Descriptive bivariate regressions: Functional characteristic Vs Linearity	**R^2**
Average groove depth v Linearity	0.581
Average wave power v Linearity	0. 431
Multicolinearity check	
Average groove depth v Average wave power	0.397
Inferential multivariate regression	
Groove depth and wave power v linearity	0.813

Table 1. Diagnostics for the descriptive bivariate and inferential multivariate regressions of seagrass linearity against groove depth and wave power, individually and collectively.

The groove depth and linearity bilinear regression gave rise to positive residuals on the west side of the atoll and negative residuals on the east side, approaching unity in the southeast. Comparison of the groove depth and wave power model revealed both variables to be at a maximum on the southeast region of the atoll. The east side is subject to high energy levels relative to shallow groove depth, whereas the west side has deeper grooves and lower energy in relative terms. The combined influence of adjacent groove depths and incident wave power explained 81% of the variation in seagrass patch linearity across the overall habitat map.

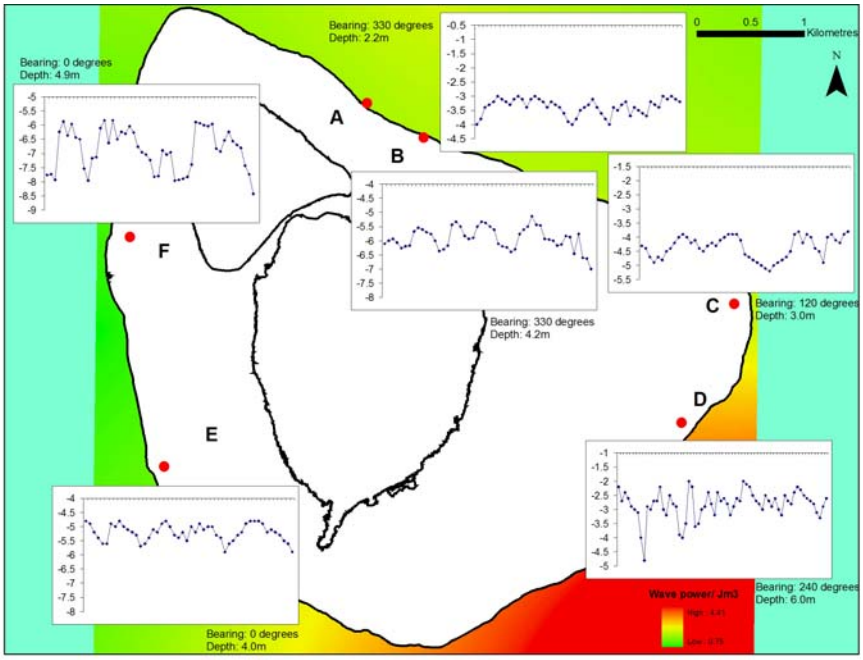

Figure 2. Analysis sites at Alphonse. Profiles of adjacent spur and groove, superimposed onto the wave power model. Each spur and groove profile represents a 50m transect across the given depth and bearing. Incident wave power ranges from 4.41-0.75Watts per cubic metre.

The estimated regression function reads:

$$\textit{Patch linearity} = -0.234 + 2.584\times10^{-3*}\textit{wind factor} + 3.93\times10^{-3*}\textit{groove depth}$$

T-test values were 9.337 and 12.429 for the mean wind force and groove depth respectively (561 degrees of freedom; $p<0.001$), suggesting it to be highly likely that the estimated coefficients are different from zero. Diagnostics indicated compliance with the assumptions of multiple regression.

Discussion

In general, seagrass patches on the reef flat are more linear than the overall habitat mosaic at Alphonse (0.2). Linear morphologies are likely a patch scale response to the hydrodynamic setting on the reef flat; such bed forms have been observed for *Posidonia australis* (Cambridge 1975), *Cymodocea nodosa* (Marbà and Duarte 1994) and *Zostera marina* (Fonseca and Bell 1998). Alternate dark and white stripes were also noted on aerial photographs of Mayotte Barrier Reef (Guilcher et al. 1965). This is in accordance with descriptions of shallow seagrass beds on Indian Ocean reef flats as "stripey zigzag" patterns (Den Hartog 1971).

Given these linkages, the spatial pattern of linearity among seagrass patches can be linked to the absolute amount of energy reaching the coastline as a result of wind-driven surface waves, as confirmed by the bivariate regression (R^2=0.43). The plan-view pattern is one of abundant alternating linear patches of seagrass and sand in the Southeast and Northwest of the atoll, with lower values in the Northeast. The amount of energy reaching a shore is influenced by water depth, which is controlled by wave-setup and tides. Setup, the rise in mean water level above the still-water elevation of the sea due to waves breaking, is a significant determinant of reef-top sediment transport (Komar 1976). Experimental results have found wave setup and flow to increase with increasing off-reef wave height and period, and with decreasing reef top water depth (Gourlay 1996). Tides operate at a lower frequency and determine the geomorphic work that can be carried out on reef flats in two ways: through unidirectional currents with a diurnal tide frequency and by tides modulating reef flat wave energy through water level. At low tide, waves break on the reef edge and no significant energy is propagated across the reef top. However, at high tides, depth-limited waves are able to propagate across the reef-top. Such a distinction is important at Alphonse, where the ~2m tidal range coincides with the height of the reef crest.

Collectively, setup and tides separate out the influence of *(i)* wind-driven surface waves, and *(ii)* subsurface currents. This separation, along with the absence of multicolinearity between the explanatory variables of wave power and groove depth, justifies the inclusion of two functional drivers operating at different depths of the water column. The geographical distribution of residuals from the groove depth vs. linearity bilinear regression revealed spatial structure that suggested additional independent

variables to have been omitted from the model would have elevated values relative to groove depth on the east side of the atoll and lower relative values in the west, as was the case with wave power. The multivariate regression encompassed processes that differed in the nature of their influence on the independent variable, as evidenced by the greater level of explanatory power of the multivariate model.

The comparison of the distribution of patch linearity to adjacent spur and groove morphology may be explained by the transformation of energy from the outer to inner sections of the reef complex. Spurs contribute to energy dissipation through the action of bottom friction against subsurface currents on the upper terrace. Frictional attenuation over spurs lowers the energy available for particle entrainment, inducing deposition closer to the reef crest. In areas adjacent to grooves, lower lying topography and a smaller surface may promote extended entrainment, producing sand tongues that extend further onto the reef flat. An energy attenuation profile could therefore be established for landward-moving reef flat water that reflects the spur and groove topography further offshore.

Conclusion

Overall, it was found that spur and groove morphology (as measured by groove depth) and incident wave power had a moderate influence on reef flat seagrass patch linearity when treated independently, with greater explanatory power in a combined model.

The distinction between the two regression steps taken in this study highlights the importance of technique in landscape ecology studies. The bivariate regressions were "descriptive", in that they were carried out on patch assemblages inside analysis windows chosen to describe the apparent seagrass patterns as a representation of variation in a response variable where all covariates were not necessarily present in the model. Geographical variation in the residuals from this step was a useful basis for addition of a covariate that enhanced the explanatory power of the model and allowed the descriptive statement to be extended to the whole habitat map.

The transition from descriptive to inferential modelling highlights the importance of good representation of both structural and functional phenomena that are commonly the focus of landscape ecologists. This analysis of patches (as the fundamental unit of habitat maps produced from remote sensing data), using landscape ecology techniques demonstrates the suitability of these combined tools for the specification, development and testing of empirical models that link structure and function in marine landscapes.

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A pan-sharpening method for satellite image-based coral reef monitoring with higher accuracy

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Abstract. A pan-sharpening method for enhancing satellite imagery is proposed as the first step for building a relatively high accuracy and low cost approach for image-based analyses of coral reefs. To achieve this objective, a low spatial resolution multi-spectral image was first spatially resampled (increasing the number of pixels) and co-registered onto a higher resolution panchromatic image. Based on multiple regression analysis, brightness information of the resampled multi-spectral image was replaced with that of the panchromatic image so that all spectral density scatter-diagrams exhibit linear characteristics with slopes equal to one and *y*-intercepts equal to zero. The method was characterized by its simplicity and faithfulness in preserving spectral (i.e., color) information. Since the resampled multi-spectral pixels were independently modified by the densities of the panchromatic pixels, all information in the panchromatic image was transferred to the resulting pan-sharpened image, thus producing lossless pan-sharpening. As a demonstration, the method was applied to FORMOSAT-2 data acquired on 31 January 2007 at Ishigaki Island, Ryukyu Islands, Japan.

Key words: Lossless pan-sharpening, panchromatic, multi-spectral, spatial resolution.

Introduction

A method for observing global changes in coral reef status is required. Remote sensing is an effective tool to map and detect broad-scale changes of coral reefs. The effectiveness of the mapping and detection using remote sensing depends on the spatial resolution of the imagery, where higher spatial resolution generally result in higher accuracy (Mumby and Edwards 2002; Andréfouët et al. 2002, 2003; Yamano and Tamura 2004). However, high-resolution satellite images are typically costly to acquire and process, which often discourages their use.

One solution to the cost issue is to introduce a pan-sharpening method to transform coarser spatial resolution satellite images into higher spatial resolutions (Nishii et al. 1996; Liu 2000). This approach has the potential to provide high-spatial resolution data at a lower cost than the direct purchase of high resolution multi-spectral imagery. Pan-sharpening is a data fusion method whereby the spatial resolution of a multi-spectral image is improved by injecting information extracted from a higher resolution panchromatic band. There have been many algorithms proposed for this type of data fusion. Recently, a public contest for pan-sharpening algorithms was performed (Alparone et al. 2007). Eight algorithms were evaluated in this contest and the GLP-CBD (Aiazzi et al. 2002, 2006) and AWLP (Otazu et al. 2005) algorithms were ultimately ranked as the best. Both algorithms use multi-resolution analysis (MRA) to achieve spatial enhancement and were derived directly from the values measured by the sensor. The algorithms also use a normalization process when transforming the multi-spectral pixel values using the panchromatic information to avoid spectral distortion

For effective image analysis, pan-sharpening methods should preserve spectral information throughout the image, including both land and water areas, and reduce errors associated with noise such as breaking waves. Since complicated processing itself may cause unpredictable noise, the pan-sharpening process approach should also ideally be simple and predictable. We propose a simple pan-sharpening method based on a multiple regression analysis. In the method, original multi-spectral images are spatially resampled (increasing number of pixels) and then modified so that scatter diagrams between pan-sharpened image and the resampled original image for all spectral bands exhibit linear relationships with slopes equal to one and *y*-intercepts equal to zero.

Material and Methods

A set of FORMOSAT-2 (Liu 2006) images acquired on 31 January 2007 at Ishigaki Island, Ryukyuu-Islands, Japan were used to demonstrate the proposed

pan-sharpening method. FORMOSAT-2 provides a total of four visible-near infrared multi-spectral bands at 8 x 8 m^2 spatial resolution and a panchromatic band at 2 x 2 m^2 spatial resolution. The panchromatic band covers the same equivalent spectral range as the four visible-near infrared bands.

Pan-Sharpening Model

In the proposed method, pan-sharpening is accomplished by replacing brightness information estimated from the original lower resolution multi-spectral (MS) bands with values derived from the higher spatial resolution panchromatic band. This process is performed while preserving the spectral information for subsequent image classification and analysis. Figure 1 illustrates how pixels for a single original MS pixel correspond with the 16 PAN pixels, and the resulting 16 pan-sharpened MS pixels. As shown in Fig. 1, the spectral reflectance values of the original MS are written as r (red), g (green), b (blue) and n (near infrared), and the corresponding panchromatic reflectance values are P_1 .. P_{16}. We regard the pan-sharpening problem as calculating the values r_i^P, g_i^P, b_i^P, n_i^P (i = 1 .. 16) as a function of a brightness estimator y, which is formulated as

$$y = f(r, g, b, n, P_1, \cdots, P_{16}). \tag{1}$$

In an early formulation of this algorithm (Gillespie et al. 1987), y was calculated simply as

$$y = (r + g + b)/3. \tag{2}$$

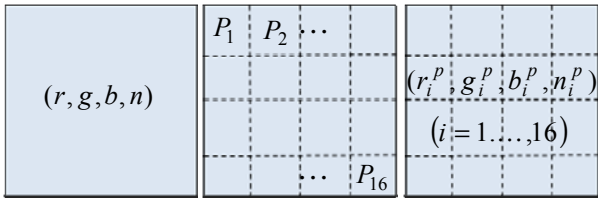


Figure 1: (a) Original MS pixel, (b) PAN pixels, and (c) pan-sharpened MS pixels.

Eq. (2) indicates that the brightness y is estimated by using just the lower resolution image, where the sum of r, g and b is a rough approximation of overall brightness. Liu used a more sophisticated function (Liu 2000) with y calculated as

$$y = \frac{1}{N}\sum_{i=1}^{N} P_i, \tag{3}$$

where N is the number of high resolution pixels corresponding to the lower resolution pixel. Liu's estimator was thus calculated using only the high resolution image. Once the estimator is obtained in the Gillespie et al. (1987) or Liu (2000) methods, the pan-sharpened values r_i^P, g_i^P, b_i^P, n_i^P (i = 1 .. 16) are calculated as

$$\left.\begin{aligned} r_i^p &= \frac{r}{y} P_i \\ g_i^p &= \frac{g}{y} P_i \\ b_i^p &= \frac{b}{y} P_i \end{aligned}\right\} (i = 1, \cdots, 16). \tag{4}$$

Since Liu's method is a form of a high pass filter, it works particularly well in photo interpretation. Both of the above methods, however, do not exactly preserve the spectral characteristics of the original multi-spectral image. This is evident when observing the scatter diagram of the original low resolution image and resulting pan-sharpened image does not have a linear relationship.

The proposed method is designed so that the pan-sharpened versus original density scatter diagram gives a perfectly linear relation with slope equal to one and a zero y-intercept. The method is a generalized algorithm that is equally applicable to both land and water applications. In this method we calculate the brightness estimator y as

$$y = a_0 r + a_1 g + a_2 b + a_3 n + a_4. \tag{5}$$

This model is derived from the fact that the panchromatic band covers the same spectral range as the visible (red, green, blue) and near infrared bands combined. Since the near infrared band reflectance is high in land (non-water) areas and almost zero in water areas, two separate versions of the model are utilized to independently represent the land and water areas respectively.

In the proposed method, each original multi-spectral pixel is first resampled into N pixels (N = 16 for FORMOSAT-2) so that resampled image has the same number of pixels as the panchromatic. All of the N pixels are initially assigned the same reflectance values as the original pixel

$$\left.\begin{aligned} r_i^e &= r \\ g_i^e &= g \\ b_i^e &= b \\ n_i^e &= n \end{aligned}\right\} (i = 1, \ldots, 16). \tag{6}$$

For the resampled image, Eq.(5) is rewritten as

$$y^e = a_0 r^e + a_1 g^e + a_2 b^e + a_3 n^e + a_4, \tag{7}$$

where y^e means brightness component of expanded pixel (i.e., resampled pixel). Using Eq.(7), the pan-sharpened reflectance values are calculated as

$$\left.\begin{aligned} r_j^p &= \frac{r_j^e}{y_j^e} P_j \\ g_j^p &= \frac{g_j^e}{y_j^e} P_j \\ b_j^p &= \frac{b_j^e}{y_j^e} P_j \end{aligned}\right\} (j = 1, \ldots, M), \qquad (8)$$

where M is the total number of pixels in the panchromatic image. Thus, the pan-sharpening problem is reduced to determining the coefficients a_0, a_1, a_2 and a_4 in Eq.(7). It is evident from Eq.(8) that when y_j^e equals to P_j the pan-sharpened pixel values are statistically the same as the original.

We use a multiple regression analysis to determine the coefficients as

$$P_j = a_0 r_j^e + a_1 g_j^e + a_2 b_j^e + a_3 n_j^e + a_4 \qquad (9)$$
$$(j = 1, \ldots, M).$$

Eq.(9) is rewritten as

$$\begin{bmatrix} P_1 \\ P_2 \\ \vdots \\ P_M \end{bmatrix} = \begin{bmatrix} r_1^e & g_1^e & b_1^e & n_1^e & 1 \\ r_2^e & g_2^e & b_2^e & n_2^e & 1 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ r_M^e & g_M^e & b_M^e & n_M^e & 1 \end{bmatrix} \begin{bmatrix} a_0 \\ a_1 \\ a_2 \\ a_3 \\ a_4 \end{bmatrix}, \qquad (10)$$

or in matrix format as

$$\mathbf{P} = \mathbf{XA}. \qquad (11)$$

The coefficient vector **A** is obtained by using a generalized inverse solution

$$\mathbf{A} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{P}, \qquad (12)$$

where **X**‘ is the transpose matrix of **X** and $\mathbf{X}^{-1}$ is the inverse matrix of **X**. The pan-sharpened multi-spectral reflectance values are obtained by solving Eqs.(7), (8) and (12).

Spatial Co-Registration

The MS and PAN sensors on FORMOSAT-2 are physically separated, which produces a complex discrepancy in spatial registration between the two sensors. In order to remove this discrepancy, a spatial co-registration process (Hanaizumi et al. 1994) was applied to the FORMOSAT-2 imagery before implementing the pan-sharpening procedure. The co-registration process consisted of automated searching for common points in the MS and PAN bands, Delaunay triangulation using the identified points, and piece-wise affine transformation for removing the discrepancy and performing the co-registration. Since all of the MS bands are already co-registered, the co-registration process was simplified by first generating a single pseudo image by adding together the reflectance values from the individual *r*, *g*, *b* and *n* images. Analysis was then performed by co-registering the pseudo image with the PAN image. Approximately 1000 points were automatically identified as co-occurring in the pseudo and PAN images. Delaunay triangulation was used to generate a surface network from these points, and piece-wise affine transformation was then used to remove the discrepancy. In this approach, only the convex areas of the Delaunay triangulation were included in the co-registration process and subsequently utilized in the pan-sharpening model.

Results and discussion

The co-registration and pan-sharpening methods were applied to the FORMOSAT-2 imagery of the northern area of Ishigaki Island. The original imagery measured 4443 x 5359 pixels for the MS bands and 17321 x 19679 pixels in the PAN image. For the pan-sharpening model, all four MS bands (*r*,*g*,*b*,*n*) were used over land (non-water) and only the three visible bands (*r*,*g*,*b*) were used over water, which includes the coral reef areas of interest. Figure 2 shows the pan-sharpening results for a 3000 x 3000 pixel subset of the overall image.

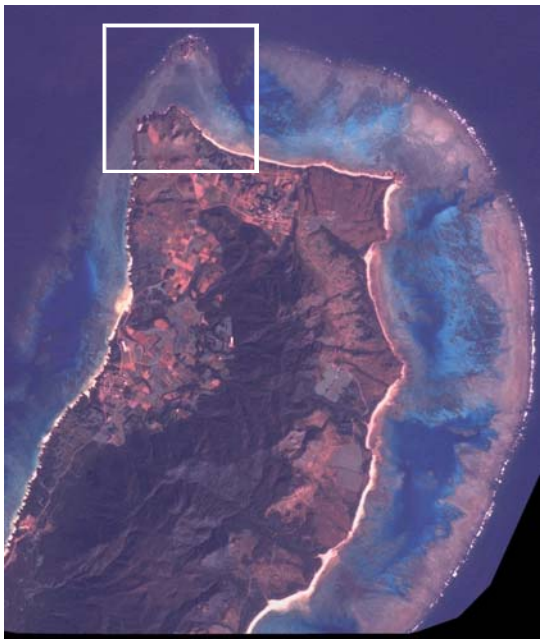


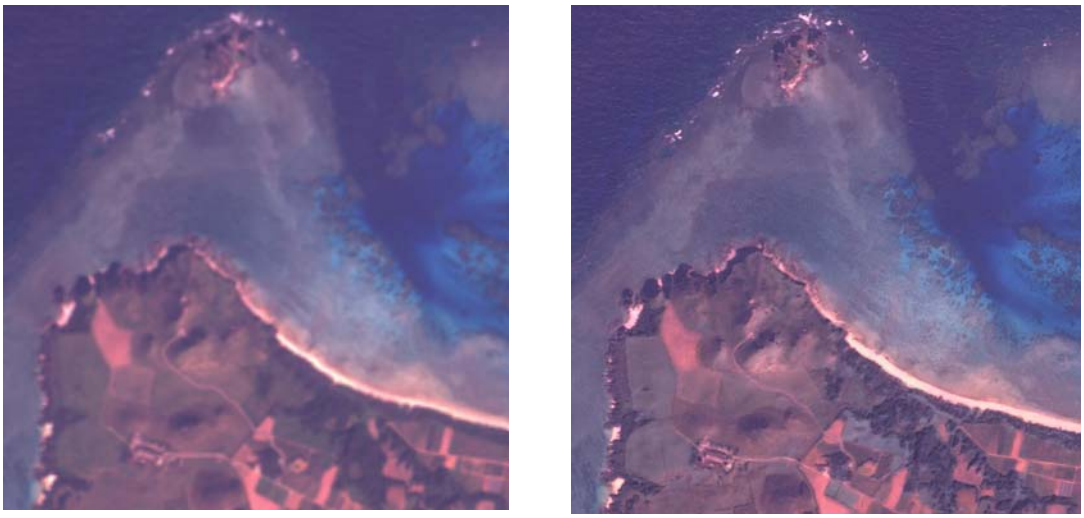
Figure 2: Pan-sharpening output for image subset, with focus area highlighted in white.

Figure 3 compares the pan-sharpening output with the original resampled image for the 800 x 800 pixel focus area indicated in Figure 2. It can be qualitatively observed that the pan-sharpened image preserves information from both the MS and PAN bands. This is because the pan-sharpening method modifies the MS pixel values using information from all bands.

In order to quantitatively evaluate the performance of the pan-sharpening model with respect to preserving spectral information, scatter diagrams were produced comparing the original resampled MS pixel values with the resulting pan-sharpened values. Ideally, this should be a linear 1:1 relationship, with a slope of one and a zero *y*-intercept. Figure 4 shows the reflectance scatter diagrams for the *r*, *g* and *b* spectral bands. In these diagrams, the *x*-axis indicates pixel values from the original MS image, and the *y*-axis is the pixel values after pan-sharpening. Further, the intensity (i.e., darkness) of each dot is proportional to the logarithmic frequency of pixels with that specific value. The red lines in these diagrams indicate the ideal 1:1 relationship. We can see that all scatter diagrams produce linear shapes closely approximating the 1:1 line. This indicates that the spectral information is preserved and that we have effectively obtained a higher spatial resolution color image of the coral reefs from the lower resolution original.

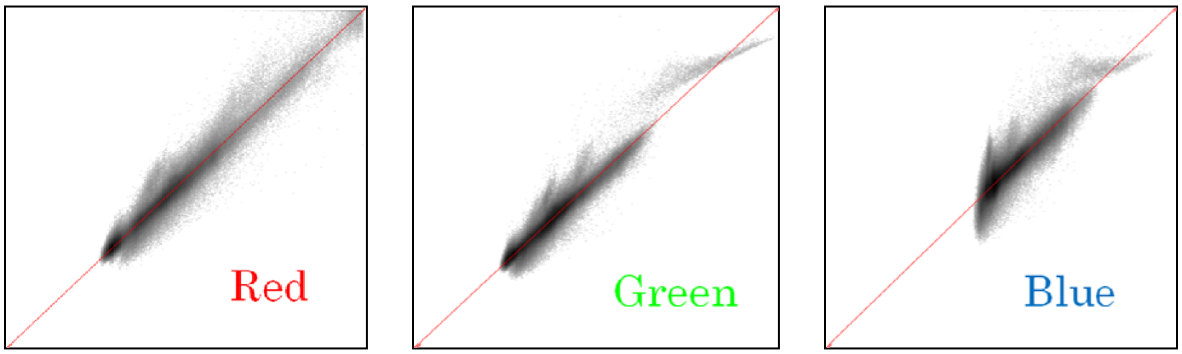
Conclusions

A pan-sharpening method was proposed which incorporates the panchromatic information without distorting the original spectral information. Application to coral reef imagery produced a visual improvement. The pan-sharpening was performed pixel-by-pixel, replacing brightness components of each lower spatial resolution MS band with higher spatial resolution values derived from the MS and PAN bands. It was also quantitatively shown that the resulting pan-sharpened image preserved the spectral (i.e., color) content of the original image. The multiple regression analysis used for this estimation is regarded as a type of density normalization between



(a) MS original color image. (b) Pan-sharpened image.

Figure 3: Comparison of pan-sharpening results with the resampled original image for a 800 x 800 pixel focus area.



(a) Red band. (b) Green band . (c) Blue band.

Figure 4: Density scatter diagrams between the pan-sharpened result (y-axis) and the original MS imgary (x-axis).

the multi-spectral bands and the panchromatic image. The model is applied over water using just the three visible MS bands, and over land using all four MS bands. By incorporating the higher resolution panchromatic information, the model also adds spatial information not present in the original lower resolution image.

The proposed method was successfully applied to FORMOSAT-2 data of Ishigaki Island, Japan. The spatial resolution of the MS bands was improved from 8 x 8 m to 2 x 2 m by implementing the pan-sharpening model. Spectral preservation performance was evaluated using scatter diagrams, which indicated results approximating the desired 1:1 relationship and thus confirmed effective performance of the model.

The authors next plan on using pan-sharpening method to monitor temporal changes in coral reefs using satellite remote sensing imagery. The development of appropriate change detection methods using this imagery is a subject of future study.

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Remote sensing for studies of the spatial distribution of coral reef fishes

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Abstract. Reef fish biodiversity is influenced by habitat, including such variables as coral cover, depth, rugosity, and the distance to the reef edge. Commercially available satellite imagery can be used to map these habitat variables, and fish biodiversity can be estimated using the resulting habitat maps. We present a case study from two reefs in Zanzibar, Tanzania, based on IKONOS imagery acquired concurrently with habitat and fish surveys. The influence of some habitat variables, difficult or impossible to measure in-situ but mappable using satellite imagery, such as habitat diversity and depth variability at a range of spatial scales, are also explored. We illustrate how well each habitat variable can be estimated using remote sensing, and how accurately a variety of models can predict the spatial distribution of reef fish biodiversity. High-resolution satellite imagery can map species richness with a Residual Standard Error of <5 species at the study site. Future refinement of habitat maps and of predictive models is expected to reduce prediction error.

Key words: Remote sensing, Habitat mapping, Reef fish biodiversity, Predictive models.

Introduction

The influence of habitat on reef fish diversity has been demonstrated in experimental and observational studies (Roberts and Ormond 1987; Friedlander and Parrish 1998; Gratwicke and Speight 2005). Habitat variables found to influence fish diversity typically include live coral cover (henceforth: coral cover), depth, rugosity, and habitat heterogeneity (Huston 1994; Chabanet et al. 1997; Jones et al. 2004). The predictive strength of these variables, individually or combined, has varied between studies (Jones and Syms 1998; Knudby et al. 2007). It is therefore prudent that any relationships be establish locally before they feed into management decisions, e.g. designation of 'high-diversity habitat' as protected.

We established habitat-fish relationships using in-situ data on a range of habitat and fish diversity variables, from two reefs in Zanzibar, one protected (Chumbe) and one un-protected (Bawe) (Fig. 1). We then assessed the feasibility of predicting spatial variation in fish diversity using remotely sensed estimations of the most important habitat variables.

Material and Methods

Three data sets were used for this study. Fish point counts were made at 93 sites on Chumbe and 51 sites on Bawe. Sites were located at a random number of fin kicks, in a random direction, from the previous site. Habitat data were also collected at these sites, as well as at 347 additional sites on Chumbe and 56 sites on Bawe, covering all major habitat types. IKONOS satellite data, from 2007 for Chumbe and 2005 for Bawe, covered both reefs.

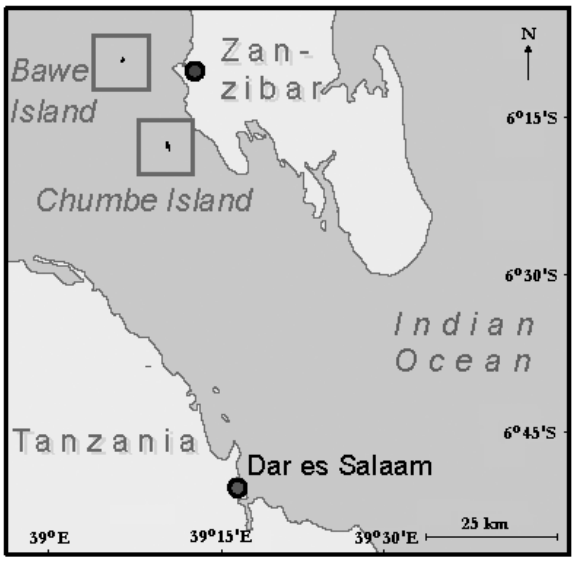

Figure 1: Study sites. The reef around Chumbe Island is effectively protected from direct human damage; the reef around Bawe Island is fished and used for dive and snorkel tourism.

Fish point counts

Fish point counts were carried out in 5m radius circles according to Bohnsack and Bannerot (1986). The location of each site was found by snorkeling in a random direction for a random number of fin-kicks from the previous site. Abundance and average length of all species were recorded.

Habitat surveys
For each site, maximum and minimum depth was measured with a dive computer, a GPS on a float was used for geolocation, coarse-scale rugosity (massive corals, patch reefs and low-frequency depth variation) and fine-scale rugosity (branching and digitate corals) were estimated on visual scales of 0-5 (Wilson et al. 2007), and substrate photos covering the 5m radius circle were processed in CPCe.

Satellite data
IKONOS images of the two reefs were preprocessed through geometric rectification, atmospheric correction with Atcor2, water column correction by calculation of Lyzenga's (1978) depth-invariant index, and sunglint removal (Hedley et al. 2005).

Data analysis
Fish point counts allowed calculation of three quantifications of the fish community: 1) species richness, 2) biomass, 3) Shannon-Weiner diversity. The biomass, based on length-weight conversion provided by FishBase (Froese and Pauly 2008), was used instead of abundance to mitigate bias in diversity calculations, otherwise caused by schools of *Chromis spp.*, often with several hundred individuals.

Depth measurements were used to calculate average depth and depth range (max depth–min depth). The substrate photos from each site were processed in CPCe (Kohler and Gill 2006) to extract percentage cover of substrate types. This also allowed calculation of coral cover, habitat diversity, and distinction between coral growth forms allowed calculation of the number of growth forms present at each site. The two reefs were processed independently.

From these sets of habitat and fish variables, individual correlations were explored. Ordinary least-squares (OLS) linear regression models were then developed to explain variation in each fish variable, and generalized additive models (GAM) were developed to account for non-linear relationships, and compared to OLS models using broken-stick transformed habitat variables. The Bayesian Information Criterion (Schwartz 1978) was used to include/exclude variables from both model types.

The satellite data were used to develop a number of products estimating habitat variables. First, a habitat map was developed, from which the habitat class and geomorphologic zone of each site could be derived. Classes were defined as: Dense coral (>40% cover), Sparse coral (5%-40% cover), Dense seagrass (>250g/m^2), Sparse seagrass (10-250g/m^2), Sand, Pavement, Algae, Deep Water (depth>15m). A linear regression of coral cover was derived from the depth-invariant index at each field site, and applied throughout the areas classified as coral in the habitat map, yielding a map of coral cover. Depth was estimated using the method by Stumpf et al. (2003), and rugosity was subsequently derived at a range of spatial scales (pixel sizes) using NOAA's Benthic Terrain Modeler with calculation from Jenness (2002). Finally the habitat map was used to derive a measure of habitat diversity at a range of spatial scales using NOAA's Diversity Calculator (Buja 2008).

Using only these remotely sensed habitat variables, new OLS and GAM models were derived, to assess the performance of IKONOS satellite imagery in predicting the fish variables.

Results
Correlations between fish variables and individual habitat variables showed several interesting patterns. Strong positive correlations with fish species richness on Chumbe were shown by both depth range (R=0.56) and coarse rugosity (R=0.66), presumably because they quantify the structural complexity of the reef at similar scales. Coral cover showed a similar correlation (R=0.58), as did the number of coral growth forms (R=0.55), with depth slightly less correlated (R=0.38). Similar results were obtained for Bawe, except for depth (R=-0.02).

Broken sticks
Analysis of the individual relationships showed that several were non-linear or piece-wise linear. Coral cover, depth, and coarse rugosity all showed positive (linear) correlations at low values, but reached a breakpoint after which further increase in the habitat variable did not lead to an increase in the fish variable.

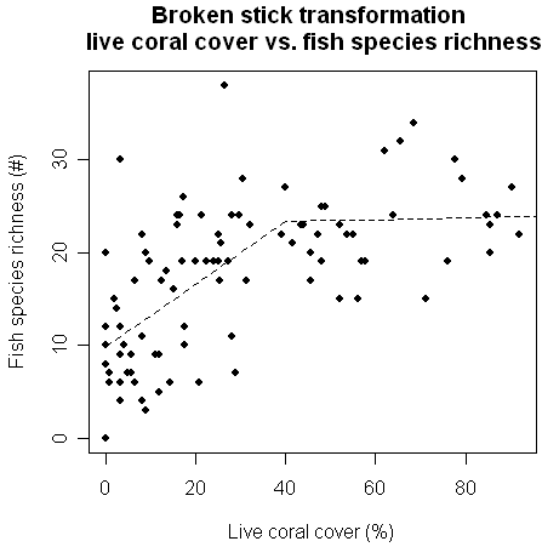

Figure 2: Broken stick transformation of the relationship between coral cover and fish species richness on Chumbe. The breakpoint at 40% coral cover was determined visually, but can be optimized to yield the best combined fit of the two segments.

Such a relationship is illustrated in Fig. 2, where additional coral cover beyond 40% doesn't lead to an increase in species richness (data from Chumbe).

When such relationships were evident, breakpoints were selected from a scatter plot, and broken stick transformations of the habitat variables were carried out. This improved individual correlation for depth range (R=0.71), coarse rugosity (R=0.73), coral cover (R=0.64) and depth (R=0.50). Variables showing linear relationships were not transformed.

In-situ model comparison

OLS models generally included variables with high individual correlations, with a few notable exceptions. Two OLS models for Chumbe included habitat diversity, which had low (non-significant) individual correlations with these variables (R=0.12 for species richness, R=0.15 for diversity). The model for biomass on Bawe included coral cover, but also three specific coral growth forms (digitate, massive and encrusting corals). All three had positive individual correlation coefficients, but negative coefficients in the OLS model. Model summaries, including GAM models and OLS models using the broken-stick transformed variables, are provided in Table 1. As expected, the GAM models produced the lowest residual standard errors (RSE) due to their flexibility.

Dependent fish variable	OLS	GAM	OLS BROKEN STICK
Chumbe: Adj. R^2 / Residual Standard Error			
Species richness (species)	0.550/5.42	0.651/**3.58**	0.648/4.79
Biomass ($g/100m^2$)	0.348/3354	0.453/**1963**	0.453/3070
Diversity (Shannon Index)	0.538/0.57	0.562/**0.38**	0.532/0.52
Bawe: Adj. R^2 / Residual Standard Error			
Species richness (species)	0.538/4.77	0.619/**3.27**	0.684/3.95
Biomass ($g/100m^2$)	0.472/995	0.621/**536**	0.507/962
Diversity (Shannon Index)	0.404/0.70	0.534/**0.47**	0.620/0.56

Table 1: OLS, GAM, and OLS broken stick models for Chumbe and Bawe. Lowest RSE values in **bold**.

Remote sensing of habitat variables

The remotely estimated habitat variables were limited to the following: coral cover, depth, rugosity, and habitat diversity.

Coral cover on Chumbe was estimated on a per-pixel basis with an RSE of 19.71 percentage points (p.p.) (R=0.66), with a non-significant improvement gained from applying a 3x3 smoothing filter to the coral cover layer (RSE=19.38p.p., R=0.67). Further smoothing degraded the correlation. Similar results were obtained from Bawe (RSE=19.72p.p., R=0.59 with 3x3 smoothing filter). Coral cover varied between 0-92% on Chumbe, and 0-83% on Bawe.

Depth was estimated to within 1m on Chumbe (RSE=1.00m) and 1.5m on Bawe (RSE=1.52m). Depth varied between 2.6-11.3m on Chumbe, and 0.6-8.9m on Bawe.

Of the two rugosity variables, only the coarse rugosity was estimated from the satellite data. This was estimated better on Chumbe (R=0.46) than on Bawe (R=0.17). On Chumbe, the correlation degraded significantly with a coarsening of the spatial scale to pixel sizes of 8 and 12m, but then improved again with pixel sizes of 20 and 50m. On Bawe, the trend was toward lower correlations with increased pixel size.

Habitat diversity was estimated very poorly, with the highest correlations R=0.14 on both reefs.

Remotely sensed coral cover and fish

The correlations that remotely sensed coral cover obtained with the fish variables is much weaker than that obtained by in-situ observed coral cover. The influence of scale reflects the accuracy of the prediction of in-situ coral cover values, peaking at a 15 meter radius (Fig. 3).

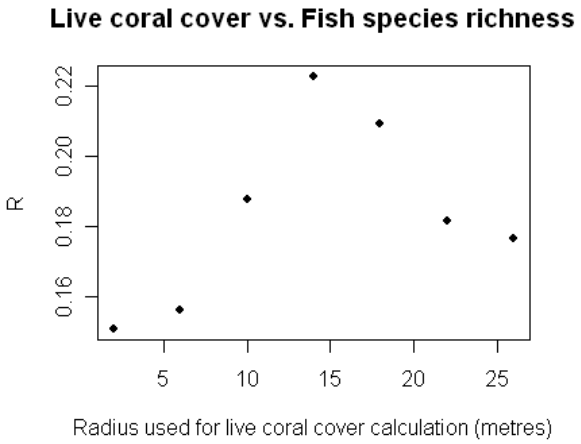

Figure 3: Correlations between fish species richness and remotely sensed coral cover at different spatial scales. Correlation with coral cover observed in-situ: R=0.58. Data from Chumbe. Optimum radius is 15 meters at Bawe, with similar correlation coefficients.

Remotely sensed rugosity and fish

Similarly, remotely sensed rugosity is correlated much weaker with the fish variables than either coarse rugosity (R=0.40, Bawe) or fine rugosity (R=0.37,

Bawe) measured in-situ. Remotely sensed rugosity obtains the highest correlation with fish species richness at small pixel sizes (R=0.22 at 4m pixel size, Bawe), the correlation degrading with increasing pixel size (Fig. 4).

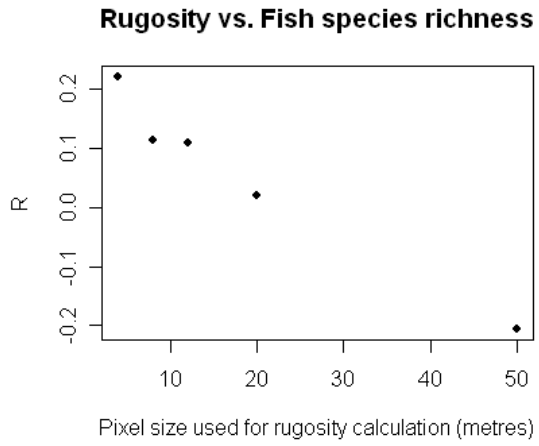

Figure 4: Correlations between fish species richness and remotely sensed rugosity calculated at different spatial scales. Correlations with other fish variables showed similar trends. Data from Bawe.

Remotely sensed habitat diversity and fish

Habitat diversity, however, showed a different trend. The in-situ habitat diversity variable had low correlations with all fish variables ($R \leq 0.14$), but when calculated on the basis of classes in the habitat map the correlations improved, reaching a peak at a radius of 30 meters ($R \leq 0.37$) (Fig. 5).

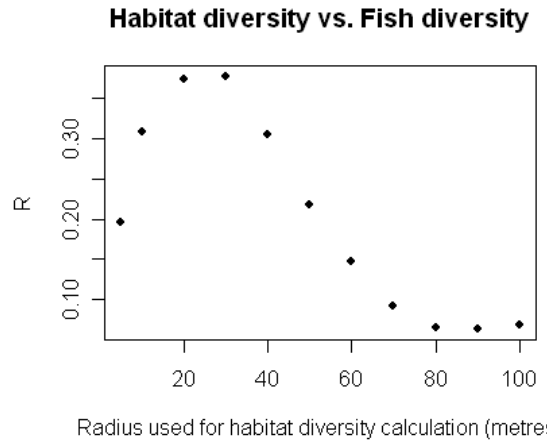

Figure 5: Correlations between fish diversity and remotely sensed habitat diversity calculated at different spatial scales. Correlations with other fish variables showed similar trends. Correlation with habitat diversity calculated on the basis of in-situ data: R=0.14. Data from Chumbe shown, data from Bawe show overall lower correlation coefficients, and two peaks at radii 10 and 50 meters.

Model comparison

The results from the OLS and GAM models, based exclusively on remotely sensed habitat variables, are shown in Table 2. A comparison reveals that models based on in-situ data generally outperform remote sensing-based models slightly, the one exception being diversity on Bawe.

Dependent fish variable	OLS	GAM	OLS BROKEN STICK
Chumbe: Adj. R^2 / Residual Standard Error			
Species richness (species)	0.491/5.76	0.458/**4.40**	0.502/5.70
Biomass ($g/100m^2$)	0.385/3255	0.375/**2109**	No trans.
Diversity (Shannon Index)	0.401/0.59	0.381/**0.43**	0.272/0.65
Bawe: Adj. R^2/ Residual Standard Error			
Species richness (species)	0.397/5.45	0.472/**3.72**	0.465/5.13
Biomass ($g/100m^2$)	0.141/1269	0.248/**763**	No trans.
Diversity (Shannon Index)	0.395/0.70	0.521/**0.44**	No trans.

Table 2: Summary of OLS, GAM and broken stick models based on remotely sensed habitat variables. "No trans." indicates that none of the variables were transformed, and results therefore similar to the ordinary OLS models. Note that the "broken stick" OLS model for diversity on Chumbe performs worse than the ordinary OLS model.

Discussion

This study provides a detailed investigation of habitat-fish relationships. The non-linear nature of several of the relationships provides interesting insights into the effects of habitat variables, e.g. showing how increasing coral cover is associated with increasing species richness, but only up to a 40% cover. For modeling purposes these non-linear relationships render linear models inappropriate, and suggest the use of GAM or other non-linear models.

Remote sensing, based on IKONOS data, was able to map both coral cover and depth fairly accurately. Depth mapping with IKONOS data has been investigated in many studies, but mapping of coral cover has remained difficult with multispectral data. We assume that the very low cover of algae on the two studied reefs has contributed to coral cover being mapped with high accuracy (<20 p.p. RSE).

Our IKONOS data were unable to map rugosity and habitat diversity accurately at the spatial scales at which these variables are observed in-situ. For rugosity, the scales mappable by IKONOS (pixel size 4 meters) have weaker correlations with fish biodiversity variables than visual in-situ estimates.

Inspection of rugosity maps illustrated that beyond the smallest spatial scales, rugosity values were overwhelmingly influences by their proximity to the reef edge, which may be the reason for the improved correlations of rugosity calculated with pixel sizes of 20 and 50 meters. In addition, the image from Bawe suffered from high-frequency noise that reduced the quality of the depth estimation, and hence the rugosity calculation.

Similar to rugosity, we were unable to accurately estimate the habitat diversity as measured in-situ, using the satellite data. However, when measured at scales accessible to remote sensing, but not to in-situ observation, habitat diversity showed improved correlations with the fish variables. This highlights an important use of remote sensing in reef studies: measuring ecologically relevant variables, such as habitat diversity, at their most meaningful spatial scale, which may be inaccessible to traditional field studies.

Optimum spatial scales

Our results are somewhat similar to those reported by Purkis et al. (2008) from Diego Garcia, who found that rugosity was most strongly related to the fish community when measured at radii ≤20 meters, and habitat diversity (quantified as evenness) most strongly related at radii 40-80 meters. Pittman et al. (2007), working in Puerto Rico, similarly found rugosity most strongly related to fish species richness at scales of 42.5 and 22.5 meters (half side lengths of square window). It thus seems that the optimum scales are fairly constant between locations, suggesting that these spatial scales have an ecological basis that is similar for the fish communities studied at these three locations.

Future study directions

The influence of habitat variables will be studied in relation to the presence/absence of individual species or the abundance of families or functional groups. This may yield greater insights into their ecological meaningfulness, which is not revealed by studying their influence on aggregate biodiversity measures as presented in this paper. The analysis of spatial scales will be included in this work, in order to attempt to explain the ecological basis for the optimum scales observed in this and other studies. Regression trees will be explored as a way to deal with the non-linear relationships between habitat and fish variables. The trees will be tested against the described models, and cross-validation applied to ensure robustness of all models.

Finally, the models will be applied to prediction of biodiversity variables in other protected and unprotected areas in Zanzibar.

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Pre-Processing 2005 AVIRIS Data for Coral Reef Analysis

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Abstract. In December 2005, the Airborne Visible Infrared Imaging Spectrometer (AVIRIS) was flown over Puerto Rico and the U.S. Virgin Islands for assessment of the 2005 Caribbean coral reef bleaching event. The resulting hyperspectral imagery is being used as the foundation for evaluating coral distribution and health in these areas. Image processing for this analysis consisted of an important pre-processing phase, followed by a spectral-based benthic classification phase. A critical aspect of the pre-processing was correcting for an adverse stray-light anomaly present in the imagery. The anomaly is characterized by higher than expected radiance values near the center of each flight line accompanied by a small decay of radiance values near the left edge of each flight line. The anomaly is present in AVIRIS imagery acquired from 2004-2006 and is most noticeable over water features in the near-infrared channels. We present an overview of the entire pre-processing phase, including suppression of the stray light anomaly and the edge decay, as well as sun glint removal and atmospheric correction.

Key words: AVIRIS, stray light, sun glint, atmospheric correction

Introduction

Spectral information from corals and other associated habitat components can be detected through a clear shallow water column using remote sensing instruments. Spectral discrimination of reef bottom types (i.e., coral, algae, and carbonate sand) is possible using field spectroscopy methods (Hochberg and Atkinson 2000; Hochberg et al. 2003) as well as using spectral image analysis techniques (Goodman and Ustin 2007; Hedley and Mumby 2003; Mobley et al. 2005). However, only visible light penetrates deeply into the water column, water properties are typically variable, and the benthic surface is spatially heterogeneous. Thus, due to the spectral complexity of this environment, data from imaging spectrometers with multiple spectral channels in the visible portion of the electromagnetic spectrum, such as the Airborne Visible Infrared Imaging Spectrometer (AVIRIS), can potentially provide better maps of benthic types, compared to multispectral sensors.

Analysis of benthic composition using remote sensing requires a number of pre-processing steps to convert the measured at-sensor radiance into reflectance data. This is because at-sensor radiance represents a complex combination of absorption and scattering features from the atmosphere, spectral interactions at the air-water interface, absorption and scattering from the water column and reflectance from the benthic surface. The sensor system itself can also add artifacts and noise during the recording process. As an important first step in effective image analysis, pre-processing is utilized to remove the confounding effects of sensor artifacts, atmospheric influence, and specular reflection from the water surface (i.e., sun glint).

We present a series of techniques utilized for pre-processing AVIRIS imagery acquired in 2005 from Puerto Rico and the U.S. Virgin Islands. Output derived from these techniques is in the format of remote sensing reflectance at the water surface, which is subsequently being used to retrieve bathymetry and water properties and to classify the benthic substrate following methods adapted from Goodman and Ustin (2007). The resulting image products are being utilized in conjunction with field surveys to assess coral distribution and health following the 2005 bleaching event (Guild et al. 2008).

Materials and methods

In mid-December 2005, AVIRIS was flown on the NASA Twin Otter over sites in SW Puerto Rico and the U.S. Virgin Islands to investigate impacts from the 2005 coral bleaching event. The Twin Otter was flown at an altitude of 3.5km, producing an image spatial resolution of approximately 3.1m. A handheld spectroradiometer (GER1500, Spectra Vista

Corporation) was concurrently used in the field to acquire reflectance measurements of the water surface at three locations.

While AVIRIS has a long history of improvements while providing high-quality imaging-spectrometer data (Green et al. 1988; Carder et al. 1993; Green et al. 1993; Sarture et al. 1995; Clark and Swayze 1996; Eastwood et al. 2000; Green and Boardman 2000; Green and Pavri 2000), in some situations the data still contain unwanted features that need to be corrected. In 2004, a new foreoptics section was installed on the system. This significantly improved the signal-to-noise ratio of the instrument (i.e., increased instrument performance), particularly in the visible portion of the spectrum, but introduced a stray-light anomaly in imagery acquired from 2004-2006. The stray light is a portion of light that has entered the instrument and should be recorded at a given measurement location (i.e., pixel), but is further reflected within the instrument, becomes mixed with other light, and subsequently inappropriately recorded at a different location. The resulting anomaly is visually apparent as a "glow" of incorrect data surrounding a stripe of "good" data where the stray-light effect is not present (Fig. 1a). Because this anomaly is a function of stray light from nearby pixels, and because land features typically reflect light strongly in near-infrared wavelengths, the anomaly is stronger at the land-water boundary and most significant in the near-infrared channels (750-1300nm). Although the anomaly is minor in the visible-wavelength channels (370-700nm) (Fig. 1b), where aquatic remote sensing is primarily focused, artifacts in the near-infrared can be transferred to the visible channels via sun glint suppression and atmospheric correction algorithms. While the impact of the stray-light effect is only a few percent of the relatively stronger signal over land, it can be as much as ~40% of the significantly lower signal over water areas adjacent to land.

Pre-processing the AVIRIS imagery for subsequent benthic analysis included suppressing impacts of the stray-light anomaly, correction of an unexplained edge decay feature, suppression of sun glint and finally atmospheric correction.

The stray-light anomaly was independently suppressed for each of the affected near-infrared channels (Fig. 1c) using an adaptive line-by-line correction that differentially adjusts the left and right sides of the image. The two sides were corrected separately because the anomaly was found to be different on each side. Correction was achieved by first using a moving window (101 along-track pixels by 30 across-track pixels) to calculate the mean of the central stripe of "good" data and the means of the "glow" adjacent to the left and right of the stripe for each line. The values for the individual pixels on each side of the image were then reduced as a function of the difference between the mean of the central stripe and the mean of the respective left or right "glow", scaled by the pixel distance from the central stripe (Fig. 2).

The decay feature along the edges of each image impacted the first seventy pixels (Fig. 3) and the last pixel in each row. This limb effect was corrected by first computing the across-track column means for a designated training area within deep water in each flight line, which was assumed to be spectrally homogenous (i.e., a flat field). Scale factors were then calculated to normalize each of the column means to a single mean for the training area. The resulting scale factors were used to adjust the values of the first seventy pixels and the last pixel of each respective flight line, thus making the edge pixels consistent with the more central pixels (Figs. 1c and 3).

Sun glint was suppressed (Fig. 1d) using a spectral normalizing procedure following the Hedley et al. (2005) variation of Hochberg et al. (2003). In this method the slope of the regression line, b, between pixel values from a NIR band (750nm) and each of the visible bands, i, is computed over a training sample containing sun glint. This slope is then used to reduce the values in each visible band at each pixel location, relative to the difference between the NIR-band minimum value within the training area, L_{NIRmin}, and the location-specific NIR value, L_{NIR}. For each band i, the output pixel value, $L'(i)$, is then:

$$L'(i) = L(i) - b(i)[L_{NIR} - L_{NIRmin}]$$

Atmospheric correction was performed using Tafkaa, an algorithm developed at the Naval Research Laboratory designed to address the confounding variables associated with performing atmospheric correction for shallow aquatic applications (Gao et al. 2000; Montes et al. 2001; Montes et al. 2003). The Tafkaa algorithm includes different aerosol options (none, continental, maritime, or urban) and a number of atmospheric gaseous absorption calculations (water vapor, carbon dioxide, ozone, nitrous oxide, carbon monoxide, methane, and oxygen). The algorithm was implemented using an option to retrieve per-pixel reflectance values based on pixel-specific solar illumination angles and AVIRIS viewing geometry, which provides a more explicit solution to the atmospheric correction calculations. Output was in the units of remote sensing reflectance at the water surface.

As a final step, the imagery was georectified using the internal geometry models (IGMs) and geographic lookup tables (GLTs) provided as standard ancillary files with the AVIRIS imagery (Fig. 4).

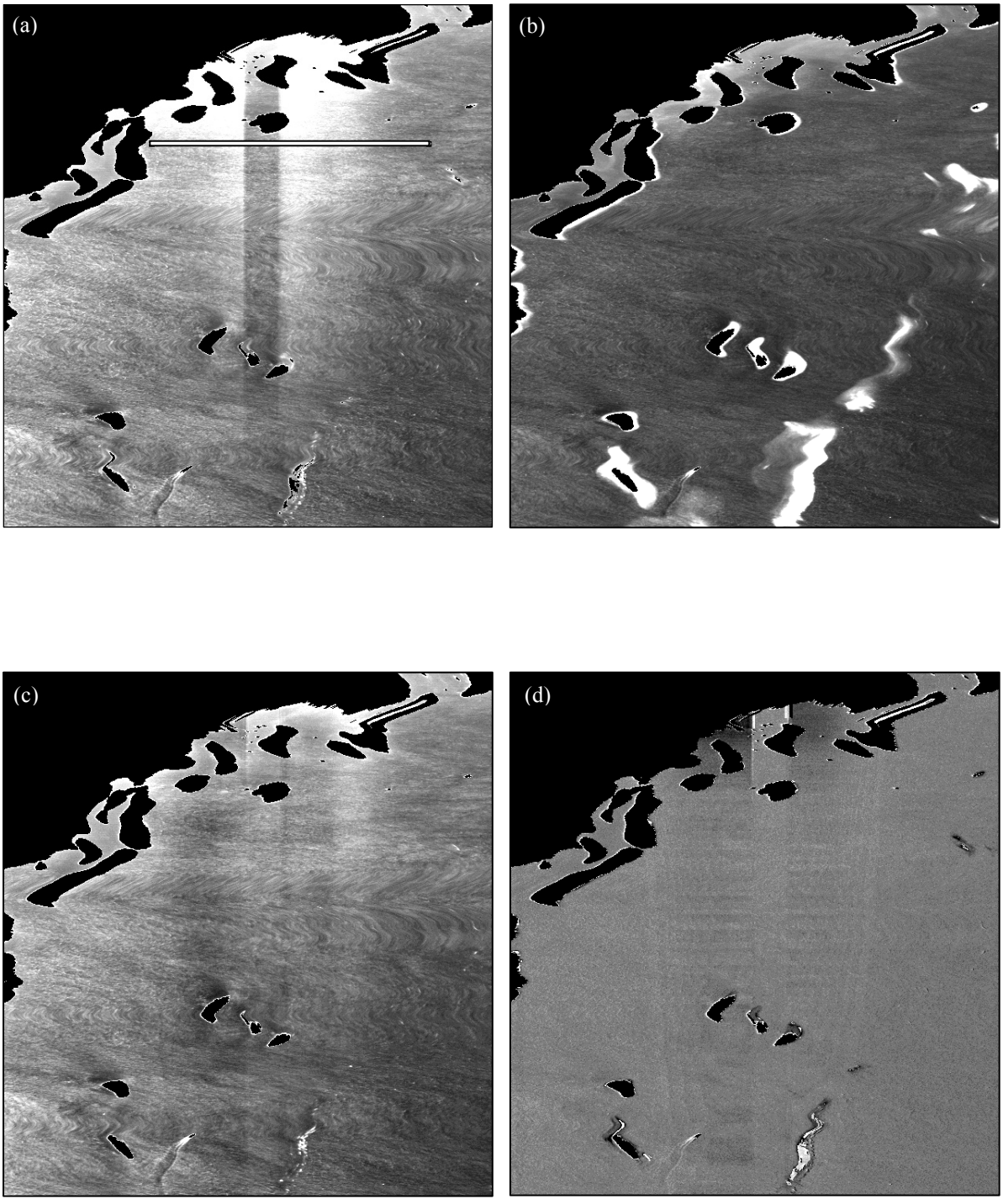

Figure 1. (a) Channel 55 (864nm, near-infrared light) within a non-georectified AVIRIS flight line subset over La Parguera, Puerto Rico. In this flight line, the stray-light anomaly was present only in the near-infrared channels. The long white box is the location of the profile shown in Fig. 3. (b) Channel 35 (672nm, visible red light). The stray-light anomaly did not affect the visible channels. (c) Channel 55 after stray-light suppression and limb correction. (d) Channel 55 after stray-light suppression, limb correction and sun glint suppression.

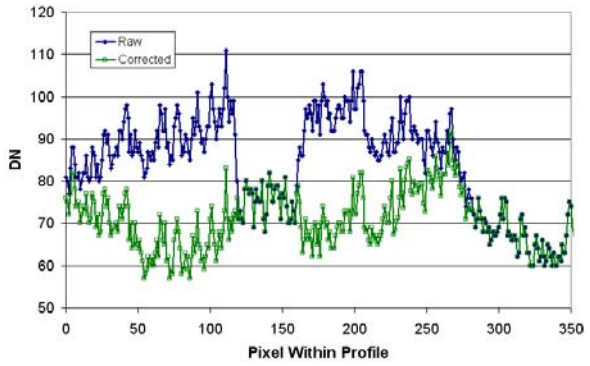

Figure 2. Cross-section profile of stray-light anomaly (from Fig. 1a). The profile shows that the digital numbers (DNs) in the relatively dark central band are similar to the background values near the edges of the swath and that the anomaly decays in magnitude away from the center of the image. The noise is due to sun glint. The corrected values were the result of adjusting the raw values to match those within the central band.

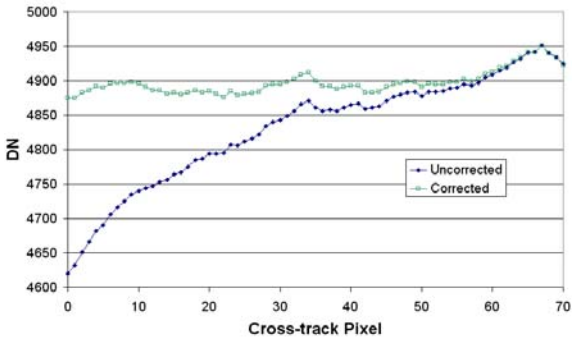

Figure 3. Deep water example of 10-channel averaged DNs within the training area (wavelengths 414-510nm) before and after limb correction. Only the first 70 pixels out of 677 are shown, demonstrating the correction on the affected side of the image.

Results

The most problematical pre-processing step was the stray-light correction, due to the variability of the effect. The magnitude of the stray-light contamination in the water depended on the proximity to land, i.e., the stray-light source, relative to the orientation of the flight line, since the amount of adjacent land determined the magnitude of the stray light. The correction estimation was further complicated by the coast line as well as the presence of patch reefs and sun glint near the coast. For the example given, Fig. 1a, and the corrected, Fig. 1c, the asymmetrical nature of the coastline and the "glow", and occurrence of small islands near the coast led to an incomplete removal of the anomaly. Generally, the post-correction values adjacent to the central stripe matched at the boundary and merged well to the magnitude of the background values (Fig. 2).

Limb corrections resulted in flatter cross-track profiles at the flight line edges (e.g., Fig. 3), which are expected for regions within the imagery that contain only water, i.e., the data are more correct. Also, this improvement should produce better mosaics when the flight lines are stitched together.

Suppressing the sun glint prior to the stray-light and limb corrections was not possible because much of the area with the stray-light contribution would have been considered glint, when the spurious values were due to a separate cause. Ideally, the sun-glint and atmospheric corrections should be performed concurrently, because they affect each other, but incorporating the atmospheric correction processing into the sun-glint suppression program was not possible.

Once all the pre-processing steps on the AVIRIS data were completed (Fig. 1) and the imagery was georectified (Fig. 4), image spectra were extracted from three locations where surface reflectance was measured concurrently in the field (Fig. 5). The AVIRIS-derived reflectance values were somewhat low compared to the field data for all three of the field sites at that location, but generally indicated good agreement between the image data and field measurements. More field data of water-leaving reflectance measurements from other locations would have allowed for better comparisons of the AVIRIS data to field conditions, but with limited equipment and a primary focus on collecting benthic reflectance measurements for habitat mapping, only limited data were available for the water surface.

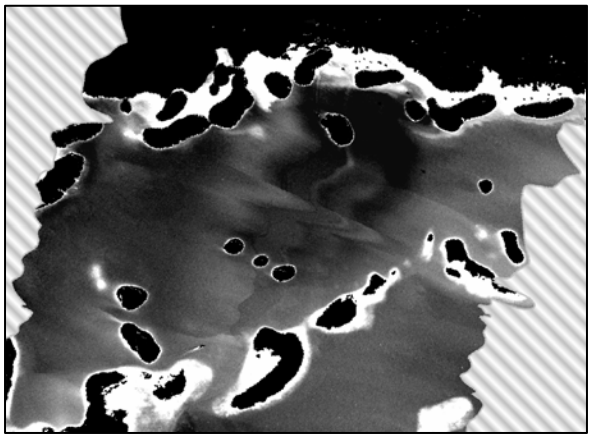

Figure 4. Channel 35 (672nm, red light) subset of sample image following complete pre-processing scheme. The wavy edges are an artifact of, primarily, the roll correction.

Discussion

As a research instrument, AVIRIS has undergone numerous changes and improvements over the years. The foreoptics section change implemented in 2004 improved overall sensor performance, but resulted in an unfortunate stray-light anomaly (note the stray-light anomaly was fixed for the 2007 flight season and only impacted data in the 2004-2006 seasons).

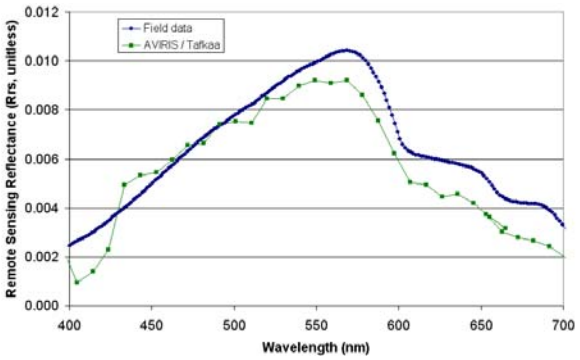

Figure 5. Image-derived and field-measured, remote sensing reflectance (R_{rs}) for one of three near-shore water sites. On average, the image-derived values were 16% lower than the field data.

We have presented an overview of the methodology used for correcting this anomaly, as well as a summary of other steps used for pre-processing 2005 AVIRIS imagery of Puerto Rico and the U.S. Virgin Islands. The corrected imagery is ready for further analysis and represents a valuable resource for evaluating coral reef conditions at the end of the mass coral bleaching event in 2005. Current analysis is focused on using the imagery to derive water properties, bathymetry, and benthic classification products (Guild et al. 2008). Results from this analysis have been consistent with additional field observations and measurements, thus lending further support to the efficacy of the presented pre-processing scheme.

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Rob Green and Michael Eastwood (NASA Jet Propulsion Laboratory) provided information throughout this analysis to help us understand the features in the AVIRIS data and their team used their engineering expertise to fix the cause of the stray light. This analysis was supported by the NASA under Grant No. NNH06ZDA001N-IDS issued through the Science Mission Directorate's Earth Science Division. The AVIRIS airborne mission was funded by NASA's Ocean Biology and Biogeochemistry Program. Goodman's work on this project was supported in part by the Bernard M. Gordon Center for Subsurface Sensing and Imaging Systems (Gordon-CenSSIS), under the Engineering Research Centers Program of the National Science Foundation (Award Number EEC-9986821).

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Habitat mapping in the Farasan Islands (Saudi Arabia) using CASI and QuickBird imagery

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Abstract. Map products derived from remote sensing technology increase our understanding and ability to manage tropical marine environments. The enhanced mapping capabilities of hyperspectral sensors are well understood; yet technology uptake, particularly for large scale tasks, has been slow. The study presented represents one of the largest hyperspectral projects to date, and paves the way towards increased use of this technology. Hyperspectral CASI-550 imagery and multispectral QuickBird imagery, was acquired over 3,168 km^2 of the Farasan Islands. In addition to the typical image processing steps, inopportune water condensation in the CASI sensors lens necessitated further processing to remove an across-track artifact. We present a simple protocol for correcting this abnormality, utilizing an abundance of optically deep water to model and correct the error. Investment in optical, bathymetric, and other supporting field data, along with the acquisition of the QuickBird imagery was vital. Data pre-processing facilitated thematic mapping with accuracy comparable to other studies, while allowing the use of spectral unmixing to discriminate coral from within algae dominated patches in shallow water (0-5 m) environments. The unmixing model proved robust, was readily adaptable to the CASI sensor and provides additional habitat information beyond the level of thematic mapping alone.

Keywords: CASI, QuickBird, remote sensing, coral reef, spectral unmixing

Introduction

The use of optical remote sensing technology to characterize reefscapes has increased in recent years. Drawing on a global dataset of reflectance spectra, Hochberg et al. (2003) showed that most reef components can be spectrally grouped into 12 fundamental categories; brown, green and red fleshy algae; calcareous and turf algae; brown, blue and bleached coral; gorgonian/softcoral; seagrass; terrigenous mud; and sand. As such, spectral discrimination is sufficient to classify basic reef components such as coral, algae, and sand, but insufficient at the species level. The vast majority of work to date has concentrated on multispectral, predominantly satellite based sensors (e.g. Landsat TM, IKONOS, QuickBird), which offer reliable data relatively cheaply. However, multispectral sensors collect data within only a few discrete bands and this spectral paucity may preclude discrimination of some habitat components. Hyperspectral sensors (e.g. AVIRIS, AISA, CASI, PHILLS), by contrast, provide higher levels of spectral detail. This may enable classification of image pixels into a greater number of descriptive classes, or facilitate deriving the relative fractional contribution of different spectral-endmembers (Goodman and Ustin 2007).

This study utilizes both Compact Airborne Spectrographic Imager (CASI)-550 hyperspectral imagery and QuickBird multispectral imagery. Using standard image processing techniques, a single integrated map product is achieved by merging classification output from the two different sensors. However, an unfortunate across-track spectral abnormality, later diagnosed as a lens condensation issue, was identified on receipt of the CASI-550 imagery. Across-track errors are typically caused by vignetting, instrument scanning, or non-uniform illumination effects, and can usually be corrected using standard processing algorithms. The across-track irregularity in this data proved more complex and necessitated a customized correction approach.

Studies to date mostly favor the descriptive class approach, where each pixel is assigned to a single thematic class, and forgo sub-pixel unmixing techniques. We examine both approaches in meeting two objectives: 1) the production of large-scale thematic habitat maps describing functionally important carbonate and sediment systems; and 2) an assessment of how spectral unmixing techniques might be applied to a CASI dataset to complement thematic information. To meet the latter objective, Goodman and Ustin's (2007) unmixing model

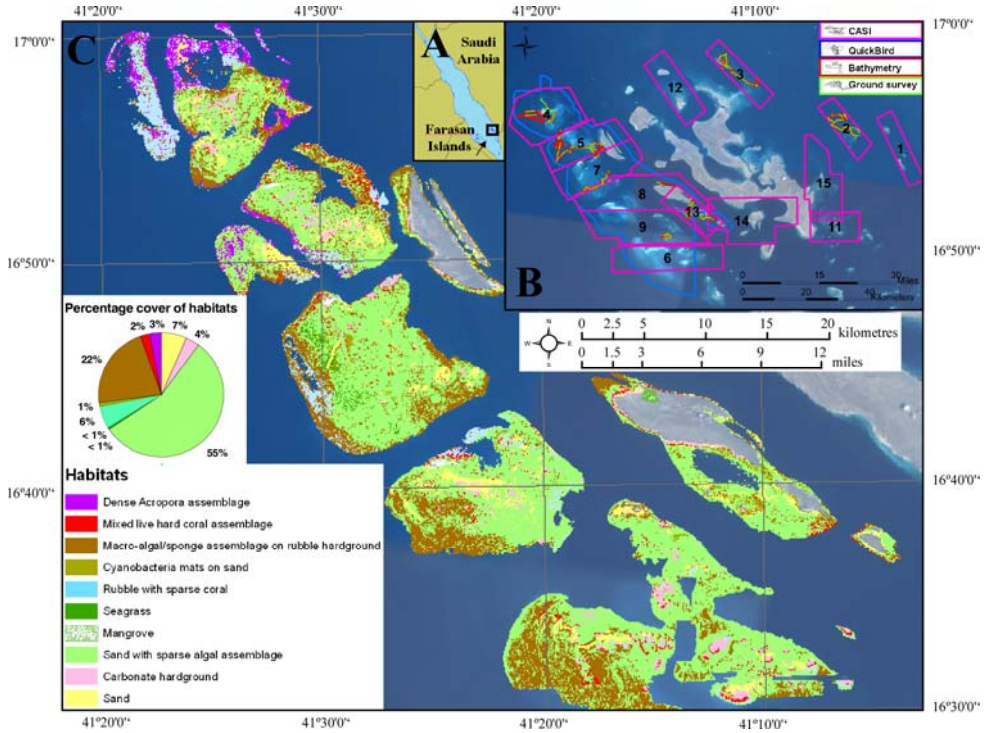

Figure 1: A) Position of the Farasan Islands, Saudi Arabia; B) Coverage of CASI imagery (magenta), QuickBird imagery (blue), bathymetric tracks (red) and ground survey points (orange); and C) Habitat map for the Western Farasan Islands produced using CASI hyperspectral and QuickBird multispectral imagery, along with percentage breakdown by habitat. Map accuracy: P_0= 82% (95% confidence intervals of P_0 are 77% and 86%), T= 79% (95% confidence intervals of T are 75% and 85%).

was employed. The approach combines a semi-analytical inversion model with linear spectral unmixing to extract sub-pixel information on sand, coral and algal composition, while simultaneously deriving information on water properties and bathymetry.

Materials and Methods

Image acquisition: In May 2006 3,168 km^2 of CASI image data were collected in the Farasan Islands, Saudi Arabia (Fig. 1A and 1B). The data presented here comprises the western bank region (Areas 4-9); approximately two thirds of the total area acquired. Data were collected at 1.5 m pixel resolution, with 19 bands assigned between 400 and 660 nm and 2 bands within the near-infrared (NIR) region, representing a non-contiguous 21 band hyperspectral dataset. Band width (FWHM) ranged from 3-7.6 nm. The instrument was mounted on a Cessna aircraft, fitted with differential Global Positioning System (dGPS), integrated to a gyroscope measuring aircraft position and movement. CASI imagery was corrected to scaled radiance prior to delivery. Augmenting this, 1,637 km^2 of high resolution QuickBird satellite data (eight scenes dating March 2004 through October 2006) were also acquired (Fig. 1B). QuickBird imagery has high spatial resolution (2.4 m pixel) comparable to CASI, but with fewer bands 4 vs. 21 and greater band width (60-140 nm).

Field survey: Field bathymetric, benthic and spectral data collection efforts were combined for maximum efficiency (Fig. 1B). Using Landsat imagery as a guide, ground tracks bisecting areas of high spectral heterogeneity, depth and exposure regimes were selected. This maximized the representativeness and diversity of habitat sampling. Single-beam acoustic sonar linked to dGPS was run continuously during the survey process, producing ~160,000 soundings, later adjusted for tidal height. A subset of 2,967 soundings were then set aside from model development and utilized exclusively for test purposes. A visual census incorporating percentage covers of major habitat contributors (corals, macro-algae, seagrass, sponges, etc) and base substrates was carried out using glass-bottom buckets, snorkel and SCUBA. Sites were chosen at random along the pre-selected ground tracks, though some spectrally distinct features were specifically targeted, producing 1604 ground truth points. A Dive-Spec underwater spectrometer (NightSea LLC) utilizing both artificial and incident light sources, calibrated against a white Spectralon panel (99% reflectance), was used to measure diffuse attenuation, marine and terrestrial endmember spectra, and deep water reflectance. Optical data described the water as intermediate between Jerlov type II and type III, indicative of a slightly turbid tropical environment.

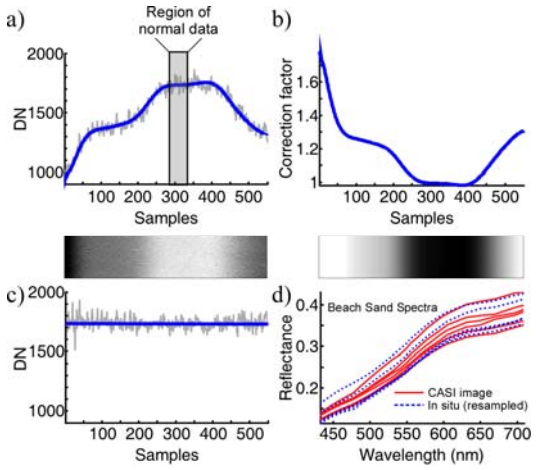

Figure 2: Across-track abnormality correction using optically deep water subset for a blue (506 nm) band. a) Across track abnormality; mean trend shown by bold line, along with region of normal data identified by field spectra comparison and image; b) Correction factor line and image; c) Corrected line and image; and d) Beach sand spectra from corrected imagery and *in situ* optics re-sampled to the CASI spectral response.

Abnormality correction: Fig. 2a illustrates the across-track abnormality, shown as the spectrum of a single band across a single line over deep water. Allowing for sea-surface and wave induced background-noise, this line is expected to have a relatively constant across-track response.

While a region of relatively flat data exists (as indicated in Fig. 2a), the spectral response drops precipitously towards both edges of the across-track array. It was further determined that this response varies across different flight-lines, thus ruling out an array sensitivity issue. Post-collection instrument analysis suggested a lens condensation problem, resulting in water pooling at the periphery of the lens. The greater affect noted towards the edges of the array, and at increased wavelengths, is consistent with this diagnosis. In the absence of a uniform solution based on the sensor, it is necessary to quantify and correct this effect based on the image data, which is difficult for areas with patchy habitats due to the inherently high spectral variation.

Optically deep water has proved useful in modeling within image spectral variation (Hedley 2005). Deep water fortunately exists in all flight-lines of the Farasan Islands. Subsets measuring 500 (along-track) × 550 pixels (across-track) were created and the across-track spectra from each subset were analyzed to determine the position of 'normal' data (Fig. 2a); typically ~70 pixels offset from centre. Mean 'normal' radiance was calculated using a 50 x 500 pixel subset from this region and divided by the mean spectrum of each sample, producing band specific across-track correction factors (Fig. 2b). The process was independently repeated for all 21 bands of each flight-line, and a correction image identical in size to the extent of each CASI flight-line created. Finally, each raw image was multiplied by its respective correction image, to normalize the peripheral regions to the spectral radiance of the central area (Fig. 2c).

Map products: Image processing followed the methodologies outlined in Fig. 3. A thematic map product was produced by first performing independent supervised classifications of the processed CASI and QuickBird imagery and then merging the results into an integrated product (Fig. 3). Field data were categorized into distinct habitat classes based on the dominant benthic coverage (see legend in Fig. 1C). A total of 800 points were used to train a maximum likelihood classification. When either sensor lacked adequate spatial coverage (or in cases where the distortion from the CASI abnormality was too high), the other sensor was used. The red and NIR bands from each sensor were used for mangrove classification and land masking, and benthic classification was based on bands from the blue-green region of the spectrum (CASI: 13 bands; QuickBird: 2 bands). A preliminary analysis was carried out for flight Areas 8 and 9 (Fig. 1B) before expanding the mapping protocols to the whole study area. Comparison of tau accuracy coefficients from 185 randomly chosen field observations suggested higher discriminatory ability using CASI in habitats shallower than 6 m (79% vs 74%) while QuickBird was the more accurate of the two sensors for habitats deeper than 6 m (80% vs 76%). Accordingly, two classifications were performed for each sensor, one classification for depths from 0-6 m and one classification for depths from 6-15 m. In the final map product, the various classifications were merged with reference to image availability, depth priority and context. For accuracy assessment of the final map, 300 points independent from the training process, distributed across the study area, were used. Most points (86%) were less than 6 m in depth, reflecting the dominance of shallow bank-top habitat.

Spectral unmixing: An additional image analysis procedure was used to examine sub-pixel habitat composition. Goodman and Ustin's (2007) spectral unmixing model was adapted to take account of the CASI band configurations. Results were evaluated using a single geocorrected flight-line with extensive ground and bathymetric data. Mean field spectra from the Farasan Islands for sand (n = 10), algae (n = 21) and coral (n = 29) were used as the spectral endmembers in the unmixing model. Derived bathymetry was compared to *in situ* depth soundings and endmember abundance maps were displayed as an RGB false-color composite for field data comparison. Accuracy was assessed using the *in situ* ground survey data.

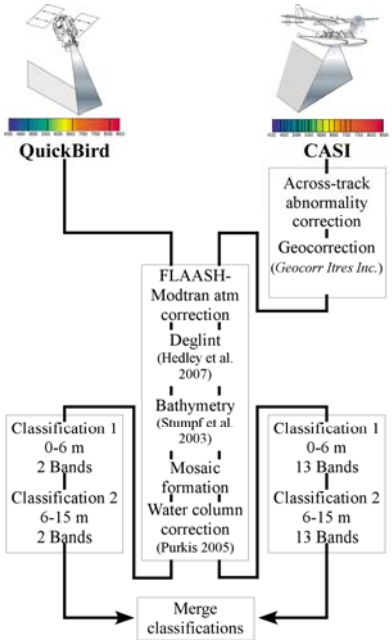

Figure 3: Processing stream for CASI and QuickBird data in producing thematic map product.

Results

The CASI across-track spectral abnormality was effectively removed using the derived correction factors, resulting in realistic spectral response across each flight-line (Fig. 2c). Background noise levels were comparable in magnitude to the raw image. Following further image processing (Fig. 3), image spectra also compared favourably to *in situ* beach sand spectra (Fig. 2d). This allowed creation of a radiometrically consistent mosaic for the study area. The method was successful in all bands for the majority of flight-lines; however, in 5% of cases, line correction was not possible as a flat region of 'normal' data could not be identified. These flight-lines were excluded from further analysis.

The combined sensor approach (Fig. 3) had a good overall accuracy (tau coefficient = 79%), mapping a total of 10 habitat classes. The western Farasan Islands are predominantly sedimentary in nature with 55% of benthos comprised of sand, or sand with sparse algae. Other areas include macro-algae/sponge atop rubble hardgrounds (22%); and rubble with sparse coral cover (6%), often intermixed with dense *Acropora* areas (3%) in the north-west. With the exception of *Acropora*, coral was only classifiable as a mixed assemblage. This comprised only (2%) of the substrate, mostly located in microatoll formations of 1-20 m diameter. Applied to Quickbird data the bathymetric model of Stumpf et al. (2003) compared favorably with actual soundings to a depth of 15 m (r = 0.91, p = <0.001, n = 2,967).

Application of the Goodman and Ustin (2007) unmixing model to the test CASI line was successful overall for investigating sub-pixel habitat composition. The comparison of predicted versus true depth (Fig. 4a) shows accurate depth determination to ~4.5 m (r = 0.83, p = <0.001, n = 161). Spectral unmixing within this depth threshold was also successful (Fig. 4b), having a high apparent overall accuracy of 76%. Small macroalgae patches (bright green) within a sand matrix (bright blue) are clear, as are transition pixels at fuzzy patch boundaries. Of particular note is the correct identification of small but abundant coral colonies at sub-pixel percentage cover, verified by field survey.

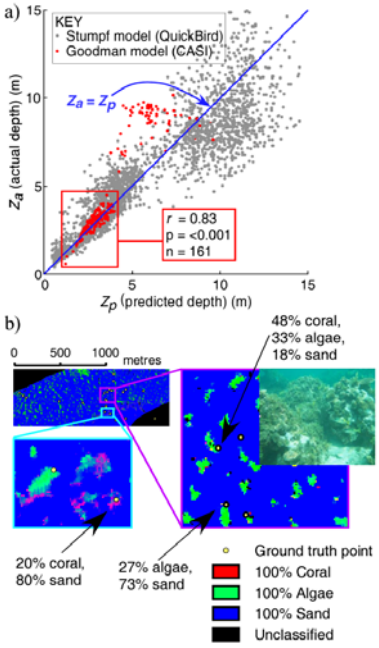

Figure 4: Goodman and Ustin (2007) model applied to CASI data showing: a) Comparison of model derived bathymetry and *in situ* soundings using Pearsons correlation; results of Stumpf et al. (2003) model applied to QuickBird also shown for comparison; and b) False color composite at differing resolutions with percentage coral, algal and sand in the red, green and blue channels respectively. Image typical of patches shown in upper right.

Discussion

The across track processing method (Fig. 2) represents a novel approach to a rare (or rarely disclosed) problem. The need for this correction is an exception and not needed for most hyperspectral acquisitions. The solution is simple, and when followed by standard atmospheric and water column corrections, a high quality image mosaic suitable for habitat mapping can be produced. The method has two processing requirements: 1) image data in a raw format uncorrected for geoposition, pitch, roll, or yaw; and 2) areas of deep water over which to model the spectral artifact. In most cases, the end users of hyperspectral imagery are not directly involved with image collection or pre-processing. Imagery is often delivered as radiometric and geocorrected flight-lines or a flight-line mosaic. In our case, we were able to liaise with our imagery supplier to acquire unprocessed imagery on which to derive

a solution. Deep water is ubiquitous in our study area. This may not be the case for all study sites; however this study supports its inclusion where possible. As all lines were affected to some degree, quantifying the propagation, or not, of error through the above process is difficult. Field optical data compare favorably to fully processed imagery (Fig 2d), and the accuracy of the resultant maps lend support to our technique. Though partly automated, our solution operates on a band-by-band, line-by-line basis, and may therefore be time intensive for large datasets. Most significantly, we were able to recover data to a good standard, allaying any loss of capital investment.

The number of habitats (10), and overall accuracy (82%) compares favorably to CASI mapping elsewhere (e.g. Mumby et al. (1998) 81% for 9 classes, above 20 m depth; Bertels et al. (2008) 73% for 10 classes, above 15 m depth). Though overall accuracy may be biased towards shallow environments, our preliminary analysis suggests accuracy is likely to be >70% throughout. Bertels et al. (2008) demonstrate mapping to a higher number of classes, but there is a clear dichotomy between the number of classes mapped and overall accuracy. The different performance at depth of QuickBird vs. CASI is notable. Given the width of the QuickBird bands, signal is integrated over a greater portion of the spectrum than the selected CASI bands, which cover a more limited spectral range. While this effect is difficult to quantify, newer hyperspectral sensors record contiguous bands over a greater spectral range, have increased signal capacity and are thus expected to perform better. In the multi-sensor mapping approach presented, the use of 10 thematic classes is well suited to integration with QuickBird classifications. Though each sensor produced good classifications independently, the merged approach allowed adjustment for shortfalls in the coverage of either dataset.

Depth derived using the Stumpf et al. (2003) model showed a close relationship with true depth to 15 m. Both this model, and the Goodman and Ustin (2007) bathymetry model, were most accurate in shallow environments (<5 m). Correlation levels compare favorably to other datasets, as well as to similar inversion models (e.g. Bertels et al. (2008) (r = 0.86)). In both studies, model performance declined with depth. This is partially a function of increased light absorbance with depth, but with respect to CASI, there is also likely to be a decrease in the signal-to-noise ratio caused by the spectral artifact.

The outputs of Goodman and Ustin (2007) model indicate that spectral unmixing is both possible and accurate (apparent accuracy = 76%) in shallow environments (Fig 4b). The unmixing model concurs with field survey data in suggesting that an ecologically significant proportion of coral in the Farasan islands are located atop hardgrounds dominated by macro-algae. This is a dominant habitat across the western banks (Fig. 3).

This project demonstrates the utility of hyperspectral technology, despite encountering unexpected processing requirements due to the spectral artifact described above. Agencies often forgo hyperspectral sensors when mapping large geographic areas. However, the two approaches are complementary, providing ecologically relevant information at different hierarchical levels. Spectral unmixing of significant habitat components adds value to conventional mapping, and will lead to the better understanding, and management, of coral reef ecosystems.

Acknowledgement

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Status and monitoring the health of coral reef using Multi-temporal remote sensing - A case study of Pirotan Coral Reef Island, Marine National Park, Gulf of Kachchh, Gujarat, India

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Abstract. The health status for Pirotan reef (22°60' N, 70°40' E) located in the Gulf of Kachchh, India, has been assessed using health indicators. Indicators of Pirotan Reef health are temperature rise, increase in macro-algae, over-fishing, high sedimentation rate, human influences, and deposition of mud over reef. Field data was carried out on the reef to collect information about the status and to map benthic cover using the line transect method. Indian Remote Sensing Satellite LISS III sensor data of periods 1998, 2000 and 2005 were analysed to zone the eco-morphological zones of the reef into nine classes with an accuracy of 89% to 92% at 90% confidence level. NOAA AVHRR average monthly SST data was analyzed from 1998 to 2005 (March, April, May and June) to identify the effect of temperature rise on coral reef bleaching. Depositions of sand and mud have increased owing to the high sedimentation rate, sand mining and cutting of mangroves. Coral bleaching and stress have resulted in increased macro-algal growth. The Pirotan reef is under severe degradation as is apparent from the indicators of health. Integrated data of multi-temporal and spectral remote sensing data, SST and field data are important to asses the health of Pirotan coral reef. Although not all deleterious processes can be measured directly (e.g. overfishing), many environmental and ecological coral properties can be measured using remote sensing.

Key words: Coral reef health, multi-temporal, Pirotan Island, remote sensing, sea surface temperature

Introduction

Changes in coral reef health and vitality are sensitive indicators of environmental change. The overall coral death is owing to the thermal stress by anthropogenic global warming, new virulent pathogens, chemical pollution, macro-algal competition and high sedimentation.

A "healthy reef" can be characterized as one with high cover of live corals and short algal turfs and low cover by fleshy algae. Associated indicators of a healthy reef are thus high cover of corals, optimum cover of calcareous algae, short algal turfs, low cover of fleshy algae and good fish diversity. The indicators of a degrading reef include decrease in coral cover, increase in massive rounded corals, increase in coral bleaching, increase in coral disease and macro-algae overgrowth. So, the indicators of the healthy reef are the opposite of a degrading coral reef. Understanding the benthic community structure is central to understanding coral reef health. Measuring the percentage of live coral cover for assessing reef degradation is important indicator for comparisons among coral reef health (Burke et al. 2002).

There are also temporal challenges from the heterogeneous and dynamic variations in coral reef communities over time, requiring long time-series observations with enough frequency to determine the state of health of coral reef ecosystems. Satellite sensors have proved to be useful in providing information on reef geomorphology, and broad scale ecological information such as the location of coral, sand, algal and seagrass habitats with accuracies of around 70% (Green et al. 2000). Satellite and airborne sensors are useful for benthic substrates mapping (Bina et al. 1978) and monitoring ecological change (Zainal et al. 1993).

The sensitivity of reef corals to abnormal elevations in water temperature has been known for many years (Jokiel and Coles 1990). Large-scale bleaching episodes indicate that coral reefs are likely to be one of the first ecosystems damaged or destroyed by global climate change (Hayes and Goreau 1991).

We have evolved an indicator-based approach for monitoring health of reefs (mainly satellite-derivable indicators). The indicators tell about the damage already done, current ecological condition and early warning to the reefs. Early warning indicators are sea surface temperature anomaly, turbidity, and the onset of algae. The ecological indicators are diversity (both floral, faunal and habitat), percentage cover of live and dead corals and the damage indicators are the deposition of mud and sand on the reef, erosion, and phase shift. The non-aligned coral zone (NACZ) is on reef tops normally receiving lower wave energy, where the reef flat is commonly formed from randomly scattered ovoid coral colonies with intervening sand patches (Hopley 1982).

The overall aim of this research paper is the development of a health model for coral reef ecosystems based on remote sensing information. This monitoring system should enable not only the efficient mapping of coral reefs, but also the identification of the reefs that are most endangered. This information will significantly contribute to the progress of protecting and restoring the coral reef environments and will in this way add to the sustainable development of these valuable natural resources.

Material and Methods

The Gulf of Kachchh has an assemblage of different ecologically sensitive ecosystems consisting of coral reefs, mangroves, seagrasses, algae/seaweeds (Fig. 1). Due to its rich diversity and fragile nature, the Government of Gujarat in 1983 declared an area of about 457.92 sq km as the Marine Sanctuary and 162.89 sq km as Marine National Park. Pirotan Island is an Arabian Sea island in the Marine National Park, Jamnagar district of Gujarat state, India. It is located 22 km off the coast, consisting of mangrove, coral reef, seagrasses, algae, invertebrates and low-tide beaches, and has an area of 3 sq km. The center of the island is at 22°60'N, 70°40'E. The Marine National park consists of 42 islands. Pirotan Island is the most popular and is one of the only two islands where visitors are normally permitted.

IRS LISS III data (23 m resolution, with 4 spectral bands: green, red, NIR and MIR) of 1998, 2000 and 2005 have been used for mapping the reef in order to know its ecological status. Required sub-images were extracted from all the data sets and were subjected to geo-referencing using Survey of India topographical maps (base maps). The images were subjected to radiometric corrections and atmospheric correction in the ERDAS IMAGINE environment, prior to implementing a supervised classification. The digital number (DN) values were converted to radiance values using the standard published gain and offset values for the LISS III sensor (Nayak et al. 2003). The images were then subjected to atmospheric correction (Green et al. 2000). We have employed here the Dark Pixel Subtraction (DPS) method. High tides modify and reduce the signature coming from the reef-scape, so it is preferred to select data acquired at low tides and clear sky conditions. We used only the low tide satellite images of the study area, so we did not perform a water column correction.

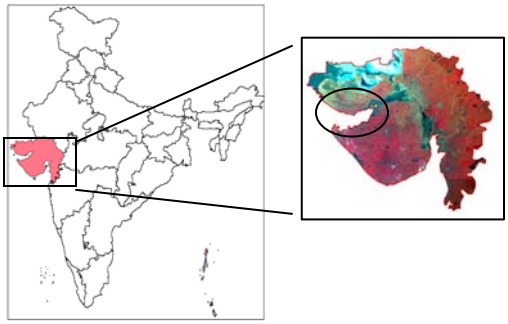

Figure 1: Location of the Gulf of Kachchh study area.

The coral reef habitats were classified using the eco-morphological classification system evolved earlier at the Space Applications Centre, Ahmedabad (Bahuguna and Nayak 1998). On the basis of the spectral properties of the reef features, supervised classification using the Maximum Likelihood classifier was performed for images of all dates. Digital classification accuracy was ascertained by estimating the overall classification accuracy and Kappa Coefficient. Error matrices were generated and the percent accuracy was estimated (at 90% confidence level) based on the number of points verified on ground and the number of failures. During the study period, we collected Line Intercept Transect data (Nayak et al. 2003). Line intercept transects of various lengths ranging from 100 m to 500 m were laid on the reef stretching from the beach seawards to the edge of the reef. After every 20 m, or at each change in zone, observations were recorded. The geographic locations of different points sampled along transect were noted using pre-calibrated Magellion GPS (stand-alone GPS) instrument with accuracy of 10 m. Substrate characteristics were recorded from each sampling location: depth of water, percentage coral, algae and seagrass cover, coral species, size of coral colonies, column of water, and sea surface temperature. We have been collecting ground data for the coral reefs in the Gulf of Kachchh every year since 1998. We collected all the ground data during low tide conditions. During low tide water depth is below 1 m and it is easy to walk on the coral reef from landward to the seaward side (along the transect).

The NOAA/NASA AVHRR Oceans Pathfinder Monthly Sea Surface Temperature images were used to calculate the maximum monthly sea surface temperature of the summer season (March, April, May and June) for 1998, 2000 and 2005.

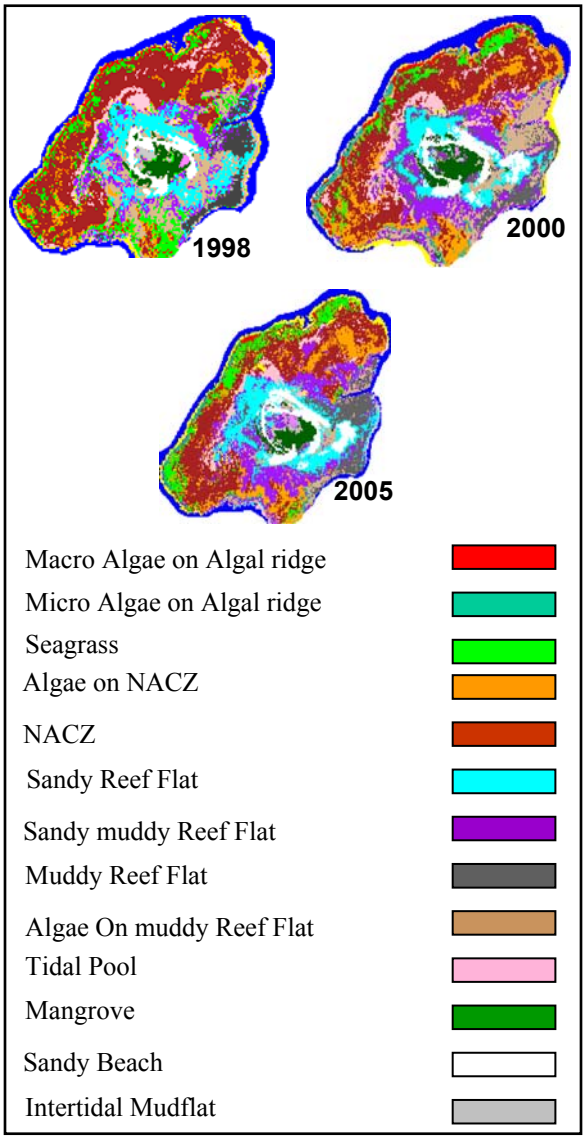

Figure 2: Eco-morphological maps of Pirotan Island reef for 1998, 2000 and 2005.

Results

Reef areas with live coral are predominantly located on the northeastern, western and southwestern areas of Pirotan Island. The entire eastern side has mud deposition. The island itself, located in the center of the study area, is mainly comprised of a horseshoe shaped sand beach, the inward side of which is occupied by mud and mangroves. The reef appears to have been an atoll in earlier times, which has been filled up with mud. The different zones classified using the satellite data were clearly distinguished: algal ridge, mud over reef, sandy muddy reef flat, NACZ, seagrass, algae on NACZ, algae on muddy reef flat (Fig. 2; Table 1). Seventy seven points were verified on the Pirotan reef for the years 1998, 2000 and 2005. There were only five failures in 1998 and 2000 and four failures in 2005. The accuracy was thus estimated to be 89% in 1998 and 2000 and 92% in 2005, all at 90% confidence level. Overall kappa coefficients were 0.85 in 1998 and 2000, and 0.89 in 2005. Major errors in misclassification were because the mangrove class got merged with seagrass, algae and algal ridge, and because muddy reef flat merged with the degraded NACZ.

The NACZ includes the live coral colonies. Live coral cover was determined by using the field data as well as the decrease cover of NACZ by eco-morphological maps during the successive years. Live coral colonies of large sizes and their high percentage are mainly confined to the seaward region of the reef. Maximum coral diversity was observed in the western area followed by northern and northwestern areas. The eastern region is most degraded as indicated by the absence of an algal ridge, presence of thick mud on the reef flat and matty algae on the NACZ.

Eco-morphological Zones	1998	2000	2005
Algal ridge	19.36	45.55	33.33
Seagrass	93.1	38.19	78.93
Algae on NACZ	56.6	122.15	127.17
NACZ	299.57	250.59	295.5
Sandy reef flat	94.00	70.3	138.97
Sandy muddy reef flat	86.7	132.51	176.53
Muddy reef flat	58.67	63.48	88.45
Algae on muddy reef flat	132.3	107.44	58.56
Sandy beach	37.93	61.95	77.29

Table 1: Area (sq km) occupied by the different zones in Pirotan Island reef of 1998, 2000 and 2005.

The areas occupied by the various eco-morphological zones are shown in Table 1. The NACZ characteristically has 25-80% live coral coverage. As the name implies this zone appears first after the algal ridge. On the reef it occupies the greatest area and is broadest in the southwest. Live corals in this zone are mainly *Porites*, *Favia*, *Favites*, *Montipora* and other species. Apart from live corals it also houses a host of faunal species. The inner part of the NACZ has more coral debris, dead coral boulders, pebbles, sand and mud, with negligible live corals. This inner part is considered in degraded condition. The NACZ was reduced in year 2000, which is attributed to the major factors such as anthropogenic influence, sea surface temperature and high sedimentation rate (Table 1).

Algae on muddy sand substrate were mapped on the inner NACZ. The algae on NACZ have been increasing severely from 1998 to 2005 (Table 1). Muddy reef flat follows the NACZ extending up to the Pirotan Island. It is uniformly distributed on all sides and the mud depositions are quite thick (almost 1-2 m in most of the places). The muddy reef flat is also increasing every year (Table 1).

Carpet and matty algae grows on the muddy reef flat. The algae are found to cover larger areas in 1998 and 2000 as compared to 2005 (Table 1). Sand reef flat surrounds the beach on the seaward side with a larger extent on the northwestern side. Mangroves occupy the inward portion from the beach. They are dense and mainly of *Rhizophora* and *Aviceenia* species. The Sargassum is mainly found in NACZ. Onset of Sargassum has shifted from September-October to early December.

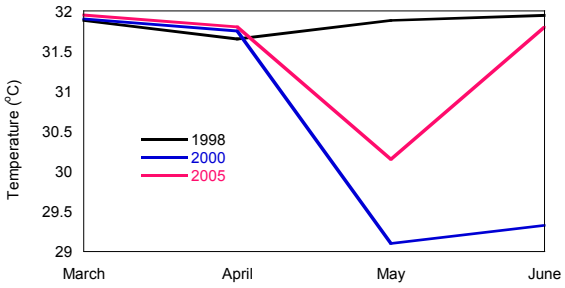

Figure 3: Maximum Sea surface temperature curve of 1998, 2000 and 2005 at the study area.

The effect of elevated sea surface temperature (SST) on the Pirotan coral reef of the Gulf of Kachchh, was monitored during 1998, 2000 and 2005. The temperature varied between 32°C and 29.2°C during summer (Fig. 3). The Pirotan reef areas in the Gulf of Kachchh are shallow, with live coral emerging from the water during low tide conditions. The reefs typically experience comparatively high temperature and seem to be acclimatized to such situations. Nonetheless, the average percentage of bleached corals (12% to 15%) during 1998, 2000 and 2005 has increased. The bleaching of corals occurs in mid March. Massive corals, especially *Porites* sp., were the first to be affected and the other dominant coral species partially/fully bleached were *Acropora* sp. and *Montipora* sp.

Discussion

The results of this study provide several useful points in terms of coral health in the context of reef degradation and disturbance. The recruitment of algal populations is especially critical during coral reef degradation, which usually involves the replacement of hard corals by benthic algae. The loss of live coral cover is often associated with a phase-shift to a system dominated by fleshy algae (Hughes 1994). In the Pirotan reef system the macro-algal (Sargassum) cover on the live coral is increasing every year. During the replacement, algal recruitment is a key but under-recognized step in the invasion and colonization of adult algal populations (Bell and Elmetri 1995). The *Sargassum* on the Great Barrier Reef (GBR), can and will colonize live corals, if conditions such as nutrients or low herbivory are suitable. Available evidence suggests that macroalgal blooms generally will inhibit corals, but also that the mechanisms and outcomes of the interaction vary with different circumstances and life stages. Decline in coral abundance often coincide with increases in macroalgal abundance (Connell et al. 1997). However, other evidence shows variable outcomes of interactions between corals and macroalgae. Corals may also competitively inhibit macroalgal growth (De Ruyter van Steveninck et al. 1988). During the recent massive bleaching on the GBR (early 1998), experimental removal of *Sargassum* canopy showed that the canopy actually protected understory corals from bleaching (Jompa and McCook 1998).

Human impacts, on the other hand, are usually chronic and they degrade water and substratum quality, thus retarding recovery (Wolanski et al. 2003). The concentration of suspended mud, and extent of stickiness and flocculation, can synergistically affect reef benthos organisms after short exposure (Fabricius and Wolanski 2000). In the present study site, the muddy reef flat is increasing due to the heavy discharge of sediments because of human impacts, such as harbor dredging, sand mining, mangrove cutting and overfishing (Nayak et al. 1989; Bahuguna et al. 1992).

River Indus is the major source of the suspended sediments in the Gulf of Kachchh (Deshmukh et al. 2005). Total coral cover was reduced because of the high sediment influx due to cutting mangroves and sand mining (GEC 1997). The eastern side of the gulf exhibits vast areas of dead corals giving a clear indication of mass mortality. Sedimentation is a major controlling factor in the distribution of reef organisms and in overall reef development (Macintyre 1988). Sediment stress has drastically reduced the coral cover and number of species. The effects from sediment influx include partial or total burial of coral colonies, bleaching, and colonization of the coral surface by filamentous blue-green algae and sponges. The reduced light levels also result in domination of the community by deeper fore-reef coral (Acevedo et al. 1989).

Coral reefs are profoundly sensitive to even modest increases in temperature and, in the absence of acclimatization/adaptation, are likely to suffer large

declines under mid-range temperature increase (Berkelmans et al. 2004). The Gulf of Kachchh reefs showed an average of 11% bleached coral with no apparent bleaching related mortality. The incidence of bleaching was not uniform every year, in terms of area and depth, but the pattern was the same. The branching corals recovered quickly after temperature reduction, but massive corals took a longer time. Temperatures in the intertidal reefs of the Gulf of Kachchh commonly reach 36^{o}C and higher in reef areas during summer and the bleaching observed could well be attributable to normal summer bleaching related to seasonal temperature rise (Arthur 2000). It is conceivable that coral species in these intertidal reefs are adapted to such seasonal temperature fluctuations (Gates 1990). According to past study conducted in 1995, estimating benthic cover in the Gulf of Kachcch, reported between 1.2% and 1.4% bleached coral in the summer months before the monsoons (Arthur 2000). This suggests that bleaching levels reported in this survey are considerably higher than normal summer bleaching. Elevated temperatures, even below the bleaching threshold may have significant impact on coral health, retarding growth and reproduction (Jokiel and Coles 1990).

These various indicators can be easily derived from remote sensing systems, which are a cost effective monitoring approach. Multi-temporal and spectral LISS III data have been proven effective to monitor and map the coral reef and extract benthic cover information. SST data products are also very important to monitor the temperature around the coral reef areas. So, integration of remote sensing and field data are important tools to obtain the health status of the coral reef. In the next step, selected indicators will be assigned weights based on their relative importance, and used as input to a model of coral reef health.

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Comparison of *in situ* temperature data from the southern Seychelles with SST data: can satellite data alone be used to predict coral bleaching events?

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Abstract. In 2003 the Aldabra Marine Programme initiated a temperature monitoring network in the southern Seychelles. It currently involves 40 temperature data loggers located at Aldabra, Assomption, Astove and St. Pierre. The annual temperature cycle in the region is marked by a shorter period of winter lows (min 23ºC) between June and October, and extended high summer temperatures (max 30ºC) between December and April. We compare satellite sea surface temperature data from four locations with *in situ* temperature measurements at 6m, 10m and 20m depth. Typically *in situ* temperature measurements do not differ from satellite measurements by more than 1ºC, though on occasions the difference can be as much as 4ºC. SST data is most similar to *in situ* data during the winter, and least similar during the summer. This is most likely due to water column stratification during the summer which is typified by calm weather with weak winds, while during the winter strong south easterly winds reduce stratification. In this case a combination of greater stratification during the summer period, along with periods of cool water upwelling, may in some cases reduce the reliability of SST data for predicting bleaching events.

Key words: sea surface temperature, coral bleaching, southern Seychelles, *in situ* temperature.

Introduction

Satellite sea surface temperature (hereafter referred to as SST) readings are now commonly used to predict where bleaching is likely to occur (Liu et al. 2006, Strong et al. 2002). Temperatures obtained using the Advanced Very High Resolution Radiometer (AVHRR) are supplied online by NOAA's Coral Reef Watch (CRW) in the form of several near-real-time global coral bleaching monitoring tools (Liu et al. 2006). Satellite temperatures used to provide these tools have been exhaustively calibrated with *in situ* sea surface temperature readings from buoys and boats (Reynolds and Smith 1994). The correlation of sea surface temperature readings used in these monitoring tools with real coral bleaching events has also been established (e.g. Cumming et al. 2003, Liu et al. 2003 , Wellington et al. 2001a), demonstrating that they are reliable for predicting when and where bleaching is likely to occur.

The link between satellite SST's and coral bleaching, however, relies on the assumption that surface temperatures will be reflected in some way at the depths where corals live. In most cases this will be a valid assumption, although local oceanographic conditions will determine the depth to which SST readings are likely to be representative. For example, satellite SST readings taken during conditions of low wind speed tend to be less consistent with bulk temperatures due to poor mixing of the surface layer (McAtee et al. 2007). This is due to the fact that satellite SST's record the temperature from the top 10µm to 1mm of the ocean, whereas they are trained to buoy and ship readings that record bulk temperatures within the first metre or so of the ocean surface (McAtee et al. 2007). It is therefore not difficult to imagine a scenario where features such as thermoclines or upwellings could spare corals from bleaching at times when surface conditions suggest it should be taking place.

In 2003 the Aldabra Marine Programme established an array of temperature data loggers at Aldabra atoll, Assomption, Astove and St. Pierre in the southern Seychelles (Downing et al. 2003). These loggers were deployed as part of a monitoring effort following the 1998 El Niño bleaching event. In particular, it was decided that such data should be collected to better evaluate the causes of future bleaching in an area of the world where there is very little monitoring of seawater temperature. The southern Seychelles is also an area where very little temperature data is available for the calibration of satellite SST readings (Reynolds et al. 2005).

Here we compare our *in situ* temperature (hereafter referred to as IST) readings with SSTs from the same

area. These temperatures are not directly comparable as SSTs are obtained from a large area as opposed to our single point temperatures, and our temperature data are obtained from different depths. However, our objective is to compare temperatures obtained by the two methods in order to determine how different they really are, and to establish whether there is a consistent difference between SSTs and IST depth readings. This will allow us to better estimate IST bleaching temperatures from SST data alone. With this in mind we also examine temperatures from both sources during a real bleaching event in the study area that occurred in March 2005.

Methods

In situ data

In May 2003 the Aldabra Marine Programme (AMP) initiated temperature monitoring in the southern Seychelles at 9 sites at Aldabra atoll and at single sites at Assomption, Astove and St. Pierre (Fig. 1). For this study we present results for the period between May 2003 and March 2006. We shall only show results for the outer reef Site 3 at Aldabra, as results are similar for all 9 sites at Aldabra. We also present results for the single sites at the other locations.

Seawater temperatures were recorded using Onset Hobo Pro® and Tidbit® temperature data loggers (accuracy ± 0.2ºC) located at 10m and 20m at all locations, and additionally from 6m at Aldabra Site 3 and Astove. At each depth two loggers (generally one Hobo Pro® and one Tidbit®) were attached to metal stakes at approximately 50cm from the seabed. Having a second logger was found to be a useful way of verifying their stability over time, as data from the two could be compared to look for evidence of drifting. Loggers were set to record every hour (though for the first two years recording was every half hour) and downloaded at approximately 18 month intervals using Onset BoxCar Pro v4.3 software. IST data was then converted to Excel format and daily averages computed for comparison with satellite data. Daily averages were used as night-day temperature differences were very small (normally much less than 0.1°C).

Satellite data

SST data were provided by NOAA Coral Reef Watch. The satellite data used for the comparison were average values derived from a 3 to 4 day composite of night-time sea surface temperatures (SSTs) observed by the AVHRR over a 0.5 by 0.5 degree pixel. For more information on how this is computed refer to http://coralreefwatch.noaa.gov/satellite/methodology/methodology.html#hotspot.

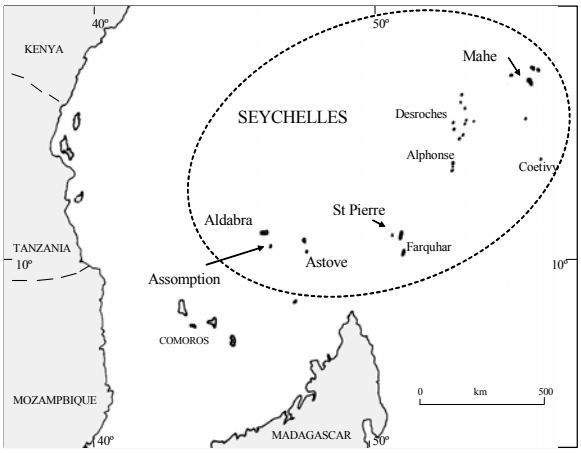

Figure 1: Location of AMP sites in the Seychelles, western Indian Ocean.

Data comparison

In order to compare satellite data with our *in situ* data we matched up our *in situ* average daily temperatures with the last day of the satellite temperature composite. From any composite data the last day is the only date provided. We used data from the satellite pixel closest to each of our sites. Where more than one pixel was available close to one of our sites we obtained an average. Midpoints of pixels used were Aldabra (09ºS 46.5ºE), Assomption (10ºS 46.5ºE), Astove (10ºS 47ºE; 10ºS 47.5ºE; 10ºS 48ºE; 10.5ºS 48ºE) and St Pierre (9ºS 50.5ºE; 9.5ºS 50.5ºE). For comparison of the two temperature sources we subtracted IST from SST readings, giving a bias reading where positive represents cooler IST than SST and vice versa.

Results

Yearly temperature cycle

Temperature at all sites ranged from summer daily averages of approximately 28ºC to winter lows of 24-25ºC (Table 1). There was little difference in temperatures at 6m and 10m, while there was evidence of thermoclines becoming established in the summer between 10m and 20m. Mean differences between the two depths in the summer were greatest for St. Pierre (mean = 0.6°C, SD = 0.4°C, Max = 2.4°C) and less at the other locations (Aldabra mean = 0.4°C, SD = 0.3°C, Max = 1.4°C; Astove mean = 0.3°C, SD = 0.3°C, Max = 1.5°C and Assomption mean = 0.2°C, SD = 0.2°C, Max = 1.0°C). At all locations during the winter period there was little difference in temperatures with depth. The general trend at all sites is one of very stable winter temperatures that do not vary much from day to day and summer temperatures that fluctuate more (see Fig. 2 for a typical example of the temperature cycle, here seen at Aldabra Site 3).

Table 1. Mean summer and winter temperatures (ºC) ± Standard Error of Mean (SEM) for AMP sites in the southern Seychelles for the period May 2003 to present (December 2006 to March 2008 depending on site). n = number of summers or winters, Max = summer maximum and Min = winter minimum temperature ± SD.

Location	Depth m	Summer average	n	SEM	Winter average	n	SEM	Mean Max	SD	Mean Min	SD
Aldabra	6	28.3	4	0.3	25.4	4	0.2	29.5	0.4	23.8	0.3
	10	28.2	5	0.2	25.4	5	0.2	29.5	0.3	23.8	0.3
	20	27.8	5	0.2	25.3	5	0.2	29.2	0.3	23.7	0.3
Assomption	10	27.9	4	0.3	25.2	4	0.2	29.5	0.4	23.5	0.3
	20	27.7	3	0.4	25.1	3	0.2	29.1	0.5	23.3	0.3
Astove	6	27.8	3	0.4	25.1	2	0.2	29.4	0.1	23.8	1.8
	10	27.8	5	0.3	24.9	4	0.2	29.1	0.6	23.2	1.5
	20	27.6	5	0.3	24.9	4	0.2	28.9	0.5	22.9	1.5
St. Pierre	10	27.9	6	0.2	26.0	5	0.2	29.3	0.4	24.1	0.7
	20	27.4	6	0.3	25.8	5	0.3	29.0	0.4	23.6	0.9

SST – IST comparison

The greatest differences between SST and IST temperatures occurred during the summer months for all sites (Fig. 3), with the transition between stratified and unstratified occurring later at Assomption and Astove.

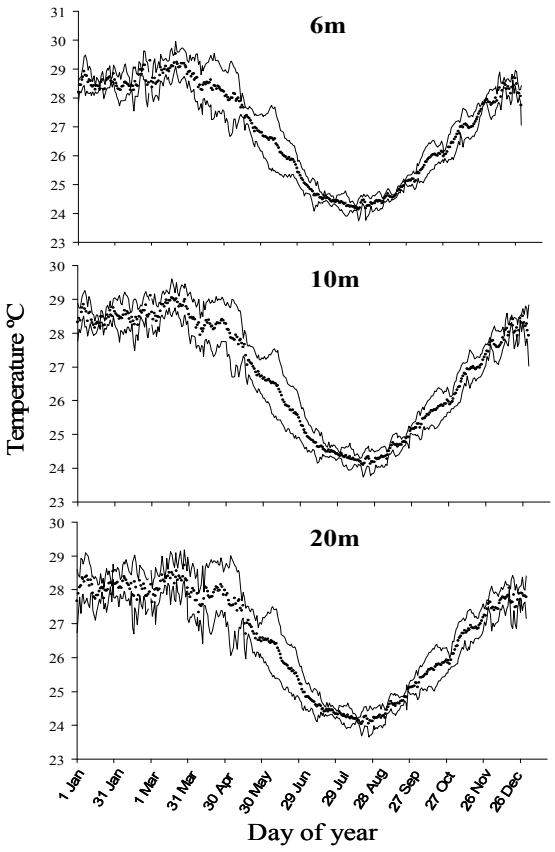

Figure 2. Example of yearly temperature cycle for the southern Seychelles based on mean daily temperature calculated from the period Feb. 2002 to Dec. 2006 for three depths at Aldabra Atoll. Dots represent mean temperatures while solid lines represent ± SD.

We examined the summer period temperatures (January to May) independently as these contrast most from those obtained by satellite. During this period the difference between SST and IST increases with depth at all sites (Fig. 4). The mean difference between SST and IST during the summer period, and at 20m depth, was highest at St Pierre (1.4ºC), intermediate at Aldabra and Astove (0.8ºC), and lowest at Assomption (0.5ºC) (Fig. 4). Points of maximum difference were reached at St Pierre (4.2ºC) and Astove (3.6ºC).

The 2005 coral bleaching event

The AMP conducted coral monitoring surveys at all sites in April 2005. During surveys we noticed diseased corals at Aldabra and Assomption, and coral bleaching at Astove. This extended from the intertidal down to over 30m depth. It included many species including members of the families Acroporidae, Poritidae, Faviidae and Agariciidae amongst others. The bleaching was, however, not intense and in many cases only parts of colonies were affected.

During this period the NOAA satellite products indicated up to 3 degree heating weeks (DHW) and 1.25ºC HotSpots for the area from Aldabra to Astove which suggests likelihood of bleaching in the period leading up to our detection of bleaching. The period of stress did, however, move in from the southeast, indicating that Astove would have been subject to a longer overall period of high temperatures.

Mean summer temperature for the region is approximately 28ºC (Table 2). We have therefore set the bleaching threshold at temperatures greater than 29ºC in line with current thinking that bleaching is likely at temperatures greater than 1ºC above the mean summer temperature (Hoegh-Guldberg 1999). IST data confirms that, while temperatures in 2005 were not excessive for the time of year, there was a

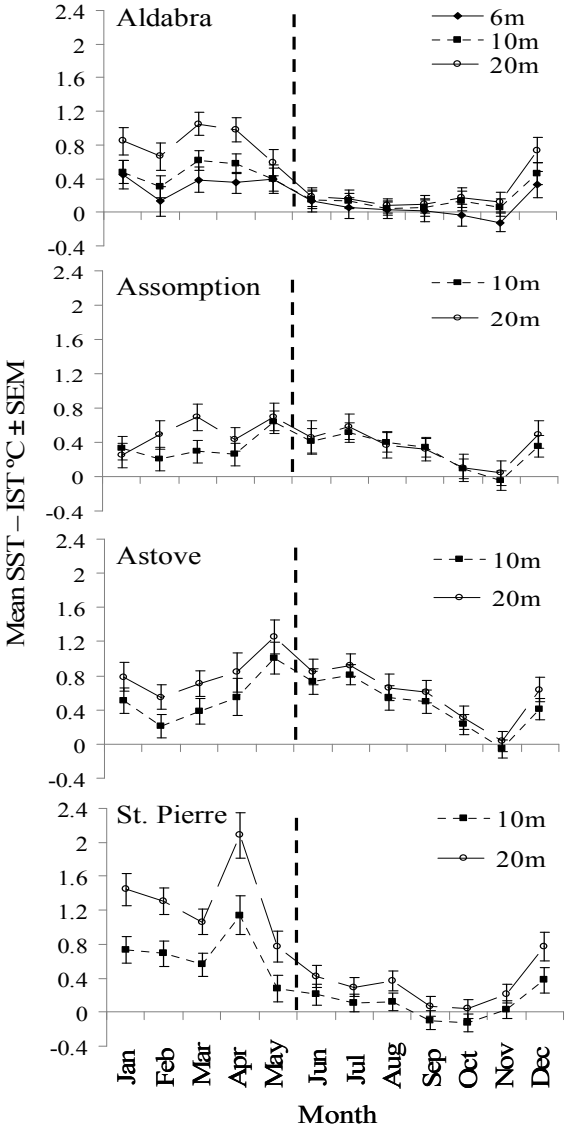

Figure 3. Mean monthly difference ± SEM between SST and IST (10m and 20m depth) temperatures obtained between May 2003 and March 2006. Dotted line represents the start of strong southeasterly winds typical of the winter period.

more prolonged period of high temperatures than for the previous years recorded. In particular, temperatures at Astove were above the 29ºC threshold on a higher percentage of days than at any of the other sites (Fig. 5). When SST data is examined it also shows the prolonged period of stress at Aldabra, Assomption and Astove. However, there is no

Table 2. Mean summer temperatures (ºC) *in situ* for all AMP sites and for SST data. n = number of summers used for estimates, SEM = standard error of the mean.

	SST	6m	10m	20m
Mean	28.5	28.0	28.0	27.7
n	16	7	17	16
SEM	0.2	0.3	0.2	0.2

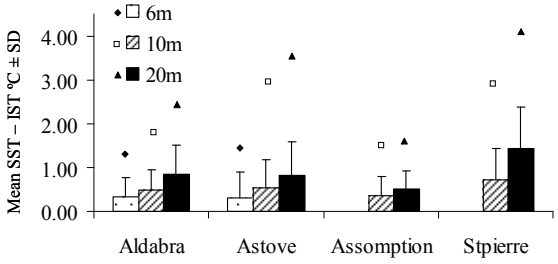

Figure 4. Mean summer differences (ºC) ± SD between SST and IST (6m, 10m and 20m depth) temperatures at AMP sites. Single symbols represent the maximum differences achieved per site and depth.

indication that Astove should be more prone to bleaching than these other locations as the percentage of days above 29ºC is similar at all of the locations (Fig. 5).

In this case SST data did not highlight Astove as a

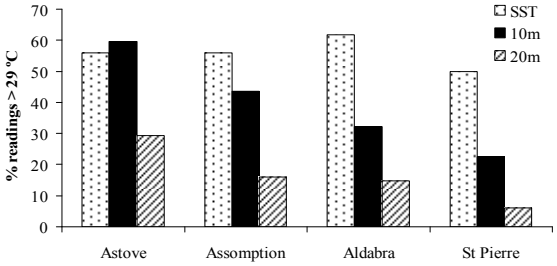

Figure 5. Percentage of days at the sea surface (SST), 10m and 20m depth with mean temperatures in excess of 29ºC at all AMP sites in the summer period leading to the detection of bleaching at Astove (01/01 to 26/04 2005).

potential risk due to several periods, particularly in February, when SSTs were lower than IST temperatures at 10m, and also, though to a lesser extent, at 20m.

Discussion

The IST temperature data we obtained from the southern Seychelles is mostly similar to SST readings provided by NOAA in spite of the differences in depths they represent. The differences between IST and SST data are negligible in the winter. The mean summer differences we obtained at 10m, with a maximum of 0.6ºC for St Pierre, are no greater than differences recorded elsewhere for *in situ* sea surface readings (e.g. Kawai and Kawamura 1997, Wellington et al. 2001b, Sreejith and Shenoi 2002).

In the winter, between June and November, strong south-easterly winds generate rough weather that leads to good mixing, thus explaining the little difference between SST and IST temperatures. Astove and Assomption are exceptions, with the winter IST and SST temperatures only equalising towards the end of winter. This is possibly due to the fact that the AMP sites at these two locations are

particularly well sheltered from south-easterly winds. The summer is typically a period of calm weather with occasional storms. Calm periods last long enough to allow the generation of thermoclines in the water column, as seen by the differences between IST and SST readings, though the temperature differences are generally not very large and the thermoclines appear to be broken down periodically by storms.

The close matching of data is also likely due to the fact that all of our locations are oceanic, with coastlines dropping off to depths in excess of 2000m very close to the shore. In essence this means that our temperature data loggers are behaving not much differently to what you would expect if they were deployed on a line in open ocean. Other SST-IST comparisons have had the confounding effect of shallower coastal waters that can warm above those suggested by SST readings (e.g. Wellington et al. 2001 b, McClanahan et al. 2007).

In spite of the good matching of SST and IST data from the southern Seychelles we must highlight the differences in the data. Generally corals below 10m will be subject to temperatures lower than those at the surface, and at levels that could mean the difference between bleaching or not. This idea is supported by our coral cover data which shows that corals below 20m were much less affected by the 1998 coral bleaching event (Teleki et al. 1999). Conversely, in 2005 we have an example at Astove of warmer water at depth than suggested by SST data. This demonstrates that local conditions can be very different to the general pattern shown by SST readings. We shall address the correspondence of SST and IST anomalies during periods of bleaching in a future publication.

The likelihood of coral bleaching is a function of the length of time that coral is exposed to temperatures above a certain threshold. Our data has shown that at these southern Seychelles sites there is a difference between SSTs and ISTs during the summer months, when the corals are most susceptible to heat-related bleaching. We conclude that while SSTs may give reasonably accurate predictions of bleaching events over a broad spatial scale, they do not always record the water temperatures to which the corals are actually being subjected. Therefore, and in agreement with the view put forward by McClanahan et al. (2007), we believe that in areas of special interest, IST data should always be gathered to more fully understand the patterns of water temperature fluctuations at the depths that really matter: not the surface, but the depths at which the corals live.

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A Model Framework for Predicting Reef Fish Distributions Across the Seascape Using GIS Topographic Metrics and Benthic Habitat Associations

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Abstract. Increased topographic complexity has been linked to increased species diversity and/or abundance in many ecological communities, including coral reefs. Several topographic metrics can be measured remotely in GIS using high resolution bathymetry, including elevation, surface rugosity, and seafloor volume within specified areas. Statistical relationships between these data and organismal distributions within mapped habitats can be used to make predictions across the entire bathymetric dataset. In this study a model framework is presented which utilizes statistically significant relationships between reef fish abundance and species richness and GIS topographic complexity measurements for samples within similar benthic habitats to create GIS-based prediction maps of abundance and species richness for the entire seascape. Reef fish associations with GIS topographic metrics were significant and varied between habitats. Model evaluation showed that patterns in the measured data emerged in the prediction data. The results allow for viewing of data trends throughout the seascape, quantification of assemblages in non-sampled areas, and statistical comparisons of areas within the region to support and guide management related decisions. This model framework can be adapted to other communities (e.g. benthic organisms) and/or parameters (e.g. diversity) that relate to topographic complexity.

Keywords: coral reef, Florida, habitat complexity, prediction, reef fish, rugosity.

Introduction

Studies linking small-scale measurements of abundances and species distributions to broad-scale seascapes are the key to understanding and predicting organismal distributions and their dynamics (Heglund 2002). Reef fish studies are often limited to small spatial scales because of logistical and economic constraints; however, viewing the data at larger spatial scales might elucidate unforeseen relationships and patterns (Sale 1998). Furthermore, the need for large-scale spatial analyses of reef fish is growing due to the over-exploitation of marine resources and the need for management and conservation of large areas (Kendall et al. 2003).

Remote sensing allows the acquisition of large amounts of data quickly and economically, providing the foundation for large-scale resource mapping and modeling. These maps are the basis upon which seascape analyses and modeling efforts are constructed (Pittman et al. 2007; Walker et al. *in press*). Previous research has shown that increased habitat complexity/rugosity positively influence reef fish abundance and/or species richness (Luckhurst and Luckhurst 1978; Gratwicke and Speight 2005). Traditional reef fish rugosity studies used an *in situ* measure of topographic complexity that is not practical on large spatial scales (>km²) (McCormick 1994); however, this is now possible by analyzing high resolution 3-dimensional topographic surfaces in GIS (Kuffner et al. 2007; Pittman et al. 2007; Wedding et al. 2008). Several topographic metrics can be measured remotely at various scales in GIS using high resolution bathymetry, including elevation, surface rugosity, and seafloor volume within specified areas.

This manuscript presents a model framework that projects the relationships between reef fish assemblage metrics (abundance, richness, etc.) and GIS topographic metrics for multiple habitats in sampled locations across the seascape. Reef fish are used as a case study to show the model design and demonstrate its capabilities. The model framework design, accuracy, strengths, weaknesses, applications and recommended uses are discussed.

Methodology

A subset of 346 stationary daytime visual fish surveys from a larger effort to acquire a baseline census of the coral-reef-associated fishes in Broward County, Florida, USA (Ferro et al. 2005) was used in this study (Figure 1). The subset was chosen on the basis of location accuracy and agreement with independent

GIS data. The fish surveys were conducted using the Bohnsack and Bannerot (1986) method between 2000 and 2002 along 54 east-west transects, each separated by approximately 0.5 km. The surveys assessed fish species, abundance, and length in a 7.5 m radius circle at each location. Each transect consisted of nine fish survey locations that targeted the eastern edge, crest and western edge of each of the three main reef tracts, yet in many cases the nearshore ridge complex (NRC) was mistaken for the Inner and Middle Reefs (Walker et al. *in press*).

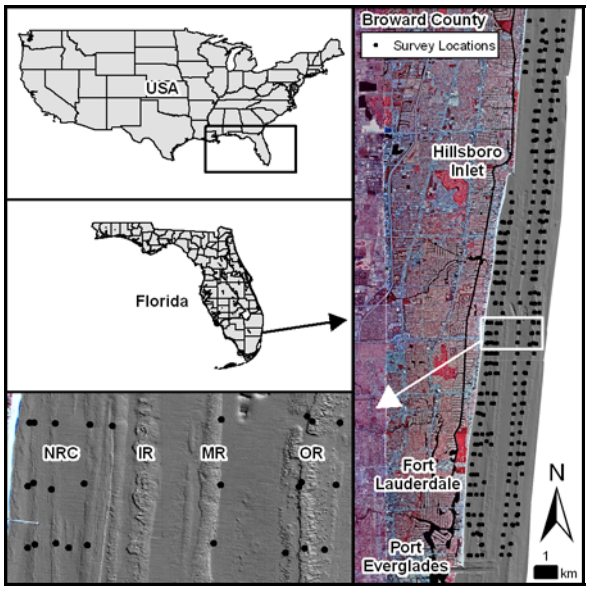

Figure 1. Aerial photo-LIDAR mosaic with the 346 point-count fish assessment sites in northern Broward County, FL, USA. NRC = Nearshore ridge complex; IR=Inner reef; MR=Middle reef; and OR=Outer reef.

GIS topographic analyses of the fish survey locations were performed in ArcGIS 9.2. Triangulated irregular networks (TIN) were created using LIDAR bathymetry for a 7.5 m radius area around each fish survey. This allowed over 12 bathymetric points per area for topographic analyses. The individual TINs were analyzed in 3D Analyst for Z min, Z max, 2D area, 3D surface area, and volume. Elevation was the positive difference between the min and max Z value. The surface rugosity index was the surface area of the TIN divided by its planar area. Volume was calculated as the space between the 3D surface and a horizontal plane at Z min.

Reef fish surveys were categorized by their location in relation to the benthic habitat characterization of Walker et al. (2008). Some habitats were excluded in the prediction model due to low fish survey sample sizes. The benthic habitats used herein were Ridge-Shallow, Colonized Pavement (CP)-Shallow, Linear Reef (LR)-Middle Shallow, LR-Middle Deep, CP-Deep, LR-Outer, and Aggregated Patch Reefs.

Analysis of variance (ANOVA) was used to analyze the data for differences in abundance and number of species per count (i.e., species richness). Abundance data (x) were log transformed using the formula $\log_{10}(x+1)$ to homogenize variance. Tukey HSD *post-hoc* tests were used to determine significance when more than two categories were examined. Linear regression was performed in Statistica 6.0 (Stat Soft Inc.) and an r^2, r, and p-value were reported for a best-fit linear regression line.

Predictions of reef fish abundance and species richness were made based on the linear regression equation of the GIS topographic measurements within each habitat. The model was created at the same scale as the fish surveys. A grid of 15 m square polygons was projected over the entire survey area. Depth, elevation, volume, and surface rugosity index were calculated for each polygon in GIS resulting in each grid polygon having individual topographic statistics and habitat characterization based on its location to the seafloor. The grid polygon topographic data values were then input into the appropriate regression equation based on the GIS metric predictor and its habitat. This generated six columns of prediction data for each grid polygon: a predicted abundance and richness for each of the three GIS metrics.

Results

A comprehensive analysis on how the fish data relate to topographic complexity is presented in Walker et al. *in press*. In summary, both abundance and richness increased with increasing topographic complexity and these relationships changed across the seascape. Richness related to topographic complexity stronger in the shallow habitats, whereas, abundance exhibited a stronger relationship in offshore habitats. *In situ* rugosity measurement yielded the best explanation of fish assemblage structure parameters, but the weaker GIS metric correlations followed similar trends. Since linear regression results varied between habitats and between GIS metrics, a separate regression equation was determined for each. Several of the relationships were not statistically significant but were included in the model for completeness.

The prediction model yielded 134,704 square polygons, each with a value for predicted fish abundance and richness using the elevation, volume, and surface rugosity values generated from the regression equations in their respective habitats, resulting in six separate prediction maps.

Linear regressions of the total measured fish abundance and richness versus the predicted values for all metrics showed statistically significant relationships ($p<0.0001$). Elevation had the highest r^2 values in both abundance and richness of the three GIS metrics, $r^2=0.27$ and 0.39 respectively; surface

rugosity had slightly lower r^2 values than elevation for abundance (r^2=0.25) and richness (r^2=0.38); and volume had the lowest r^2 values for both abundance (r^2=0.19) and richness (r^2=0.31).

ANOVA comparisons of reef fish abundance between the surveys and predictions within habitats showed only one statistical difference where volume abundance was significantly higher than the measured abundance on the LR-Middle Shallow ($p<0.05$) (Fig. 2, upper). In every other case, the predicted means were not significantly different from the measured means for each habitat. This resulted in the data trends of the empirical values emerging in most of the predictions. For example, both measured and predicted reef fish abundance were significantly lower ($p<0.05$) in the CP-Shallow than the LR-Middle Shallow, the LR-Middle Deep, the LR-Outer Reef, and the Aggregated Patch Reef.

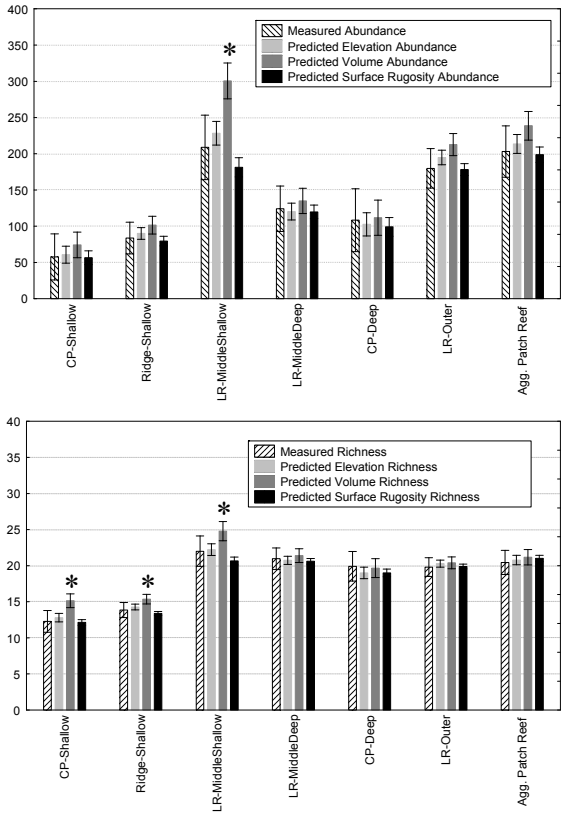

Figure 2. Measured abundance (upper) and species richness (lower) (hashed) and predicted values of reef fish by GIS calculated elevation (light grey), volume (medium grey), and surface rugosity (black) by benthic habitat. Error bars show one standard deviation about the mean. * indicates significant difference from measured abundance ($p<0.05$).

Comparisons of species richness by ANOVA between the surveys and predictions within habitats showed that the predictions did not significantly differ from the measured data with the exception of three significantly higher volume predictions ($p<0.05$) (Fig. 2, lower). In every other case the modeled data showed the same trends between habitats. For example, the CP-Shallow and Ridge-Shallow had significantly lower richness than the other habitats which did not significantly differ from one another for the measured and predicted data ($p<0.05$).

Discussion

Previous analyses of reef fish and LIDAR topography have either not attempted modeling (Kuffner et al. 2007) or focused modeling efforts on species richness (Pittman et al. 2007). The model presented herein adopts an approach to predicting reef fish distribution not previously reported. By using new technologies to project the relationship of both species richness and abundance to large-scale topographic complexity across the seascape, it provides the ability to view, quantify, and relate these predicted data.

Biological modeling involves less certainty than models based on physics or chemistry, which are derived from fundamental laws (Mitasova and Mitas 2002). The accuracy of the model presented herein relies heavily on the observed data. Although statistically relevant, the regressions showed a relatively low agreement between the predicted and measured data (r^2 = 0.27 for abundance and 0.39 for richness). This relationship was expected to be very high ($r^2 > 0.80$) since the model was developed using the same data. The output weaknesses were likely caused by the weak measured relationships between the measured reef fish variables and GIS metrics (Walker et al. *in press*). Because the initial relationship is weak, the output did not yield a high degree of accuracy. However, the comparisons between mean abundance and richness values of the fish surveys (measured data) and the predicted values among benthic habitats showed high agreement. In most of the habitats neither mean predicted abundance nor richness significantly differed from the mean measured values with the exception of volume. Hence, the empirical data patterns between habitats emerged in both predicted abundance and richness exhibiting the same trends in the data within each habitat. This suggests the model is more powerful as a comparative tool than a tool to predict absolute values in an area.

As a comparative tool, the model can provide very useful information for decisions on Marine Protected Area (MPA) placement. An MPA's location is of key importance to optimize its potential (Baker 2000). MPAs representing a full range of habitats are most effective (Carr et al. 2003) and they should contain essential fish habitat (Rieser 2000) and highly rugose areas (Friedlander et al. 2007). This model provides the information necessary to statistically compare different areas based on the organism's relationship to

topography throughout the seascape. For example, a comparison of model data between two 1 km stretches of Middle Reef shows clear quantifiable differences (Figure 3). A T-test comparison showed predicted mean abundance in area A (253.9 ± 4.5 SEM) was significantly higher than area B (178.8 ± 2.7 SEM) and area A contained significantly higher species richness (23.8 ± 0.16 SEM) than area B (21.6 ± 0.09 SEM). In this example, area A would be a better conservation area based on predicted fish data and because these data are in GIS, they can be analyzed in relation to other data relevant to MPA design and implementation.

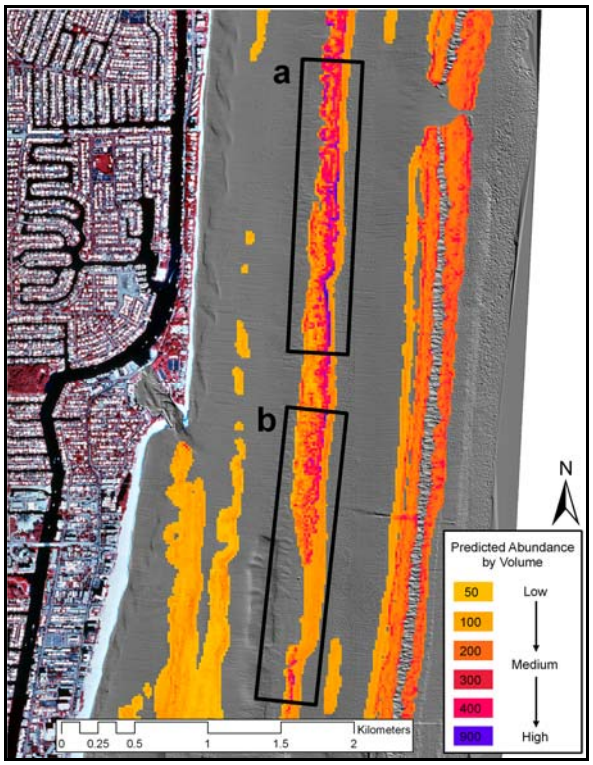

Figure 3. A map of the predicted fish abundance by volume showing two identically-sized areas used for statistical comparison. Box a contains significantly higher mean predicted abundance and richness than box b.

Bathymetry

High resolution bathymetry is some of the most valuable data to acquire in mapping submerged lands. These data, which have many uses beyond the scope of this study, were essential to mapping the benthic habitats and obtaining topographic measurements of discrete areas over the seascape. The 4 m resolution bathymetry was sufficient to map the habitats; however, it was not ideal for measuring the topographic variables at a sufficient operational scale to the fish assemblage (Walker et al. *in press*). Differences in bathymetric resolution have implications on the topographic measurements calculated in the GIS (Wolock and McCabe 2000). It is recommended that future bathymetric surveys be taken at a higher density to obtain more accurate topographic information.

Benthic Habitat Mapping

Benthic habitat mapping is an essential tool for effective management of submerged resources (Friedlander et al. 2007). Mapping the resources not only aids resource managers in the determination of mitigation for impacts, the designation for marine protected areas, and the identification of essential fish habitat, it also can elucidate previously unforeseen relationships in data brought on by the proper classification of the sample sites (Walker et al. *in press*). For Example, on a patch reef system in Biscayne National Park, FL, Kuffner et al. (2007) did not find significant differences between abundance and richness with rugosity in pooled data, but found significance when the data were split by individual patch reef. Hence, measuring changes in relationships between habitats is essential to the accuracy of prediction models.

The scale of habitat mapping can also affect the model and it is likely that a map at a finer scale would produce better results. In the current map, the area within each polygon is homogenous as described by each classifier (Walker et al. 2008). The absence of within-polygon variation might significantly underestimate the total variance of the polygonal data (Bian 1997). The variation of benthic cover within habitats could introduce significant variation in the data, obscuring other relationships (Aaby et al. 2004). Since variations within habitats (patchiness) were acoustically detected (Walker et al. 2008), it is possible that this confounded the reef fish-topographic complexity relationship.

It is recommended that benthic habitat mapping be created at the finest scale possible to include variations of patchiness within major habitat categories. This can be accomplished through high density acoustic surveys or LIDAR backscatter habitat classification (Foster et al. *in press*).

Model Adaptation

This empirical static model has been developed based on the statistical analyses of observed data enabling views of the relationship between reef fishes and their habitats on a large scale (>100 km²), allowing for statistically comparable analyses between areas based on empirical data, and thus giving statistical support to resource management decisions. Its simple design makes it highly adaptable to other uses. The framework can be used to predict any biological/ecological relationship to topographic complexity provided the bathymetry and mapping

data are of the appropriate scale. For example, it could be used to predict coral reef biodiversity via topographic complexity. The grid polygon size could be adjusted to change the scale of the model and the benthic habitat resolution could be tuned accordingly.

This system could also be taken to the next level as a spatial decision support system- a computer-based system designed to assist decision making (Corbett et al. 2002). The framework could be assembled in a user-friendly program with more automated processes and the ability to obtain instant viewable results in a GIS. Once the grid has been created and the topographic statistics calculated, fine tuning the ecological process relationship is a statistical procedure that could be self-contained in a program that would allow a user to specify the relationship (i.e. input the regression equations) and quickly view the results. This could be extremely useful to scientists studying different ecological processes and resource managers in making decisions on resource use and/or mitigation.

Future research can greatly increase this model's accuracy. Increasing the resolution of bathymetric data and habitat mapping units would eliminate several possible error sources; however, research is still needed to better understand the dynamics of how reef fish relate to topographic complexity and the other ecological factors influencing their distributions. Better understandings of the appropriate measurement scale and the scales at which reef fish operate would help to model their distributions more accurately. As these relationships are uncovered, modeling efforts using topographic complexity as a proxy for organism distribution may become more accurate.

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Seafloor characterization using multibeam and optical data at French Frigate Shoals, Northwestern Hawaiian Islands

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Abstract. Multibeam bathymetry, backscatter, and optical data collected by the NOAA Coral Reef Ecosystem Division (CRED) were used to create maps of seafloor habitats on the bank top at French Frigate Shoals (FFS) in water depths ranging from <10-100 m. Supervised classification of backscatter and optical data with user-defined classes results in seafloor maps such as hard (rock, rubble, etc.) and soft (sandy) bottom. However, they suffer from a dependence on the generally limited optical data. Uncertainties in camera sled positioning, limited availability of optical data, and user bias in the supervised class definitions suggest an alternate approach may be necessary. Unsupervised classification of different combinations of bathymetry and backscatter derivatives uses the statistical separability of the data to define unique seafloor types. The optical data are then used to define and evaluate the accuracy of the classes. A variety of methods are being evaluated to characterize benthic habitats and the resulting maps are being used to improve sampling techniques for long-term ecosystem monitoring and to guide groundtruthing operations. Future plans include using these methods to identify coral-rich and species specific environments in the Northwestern Hawaiian Islands (NWHI).

Key words: seafloor characterization, multibeam bathymetry, backscatter, Northwestern Hawaiian Islands

Introduction

Since 2002 the NOAA Coral Reef Ecosystem Division (CRED) has collected multibeam bathymetry, backscatter, and optical data as part of an effort to delineate benthic habitats of coral reef ecosystems throughout the U.S. Pacific Islands. The data provide resource managers with high-resolution maps of the seafloor and ground-truth information in water depths greater than 20 m.

Much of the mapping has focused on the islands, banks, and atolls that form the Northwestern Hawaiian Islands (NWHI) (Fig. 1), a long chain of inactive volcanoes produced by a deep seated mantle hotspot currently located beneath Kilauea Volcano (Rooney et al. 2008). Pacific tectonic plate motion slowly carries the volcanoes to the northwest away from the hotspot and they subside and become low-lying islands, atolls, flat-topped banks, and eventually guyots (Grigg 1982). Due to their remote nature, the NWHI are home to some of the healthiest coral reefs in the world and are often referred to as near-pristine coral-reef ecosystems (Page-Albins et al. 2009). It is critical that they are studied to provide baseline information for comparative analyses with degraded reef ecosystems.

Here we present results from recent efforts to create maps of seafloor substrates by applying basic image processing techniques to a combination of multibeam bathymetry, backscatter, bathymetric derivatives, and optical data collected at French Frigate Shoals (FFS) in the NWHI. These efforts have resulted in hard and soft seafloor substrate maps covering the surveyed portion of the FFS bank top. The maps are being used to determine long-term monitoring sites in the framework of a habitat-based, stratified random sampling design (Ault et al. 1999) and for continued coral reef ecosystem research in the NWHI.

Data

Figure 1 shows the bank top bathymetry data collected at FFS in 2005 using hull-mounted multibeam sonars: a 240-kHz Reson 8101 on the 8-m-long survey launch R/V *AHI* (Acoustic Habitat Investigator) and a 300-kHz Kongsberg EM3002d on the NOAA Ship *Hi'ialakai*. Both sonars provide bathymetry and backscatter data logged in the Generic Sensor Format (GSF). Vessel position, velocity, attitude, heading, predicted tides, and sound-velocity corrections are applied to the data in real-time.

The bathymetric data were edited on a swath-by-swath basis and in an area-based editor using SAIC's SABER software. Generic Mapping Tools software (Wessel and Smith 1998) was used to create ASCII grids for import and analysis in ArcGIS. The backscatter data were processed using Hawaii Mapping Research Group (HMRG) software.

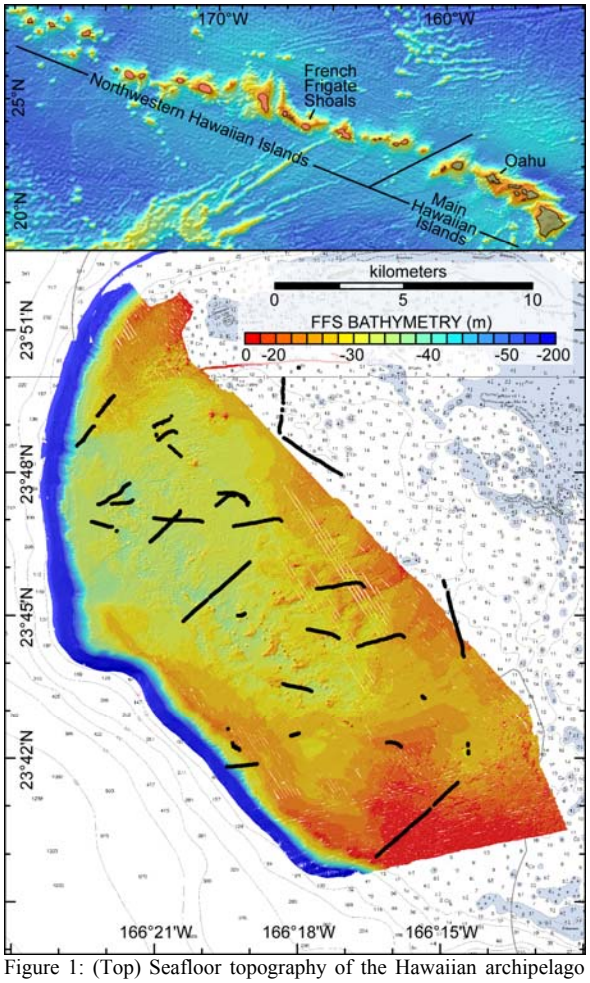

Figure 1: (Top) Seafloor topography of the Hawaiian archipelago derived from satellite altimetry and ship soundings (Smith and Sandwell 1997). The subaerial extents of the MHI and the prominent islands, atolls, banks in the NWHI and the shelves around them are outlined. (Bottom) Multibeam bathymetric data collected by NOAA over of a portion of the bank top at FFS. TOAD tracks are shown in black. The data are overlain on a NOAA NOS nautical chart where shoal depths are colored.

Optical data were collected at FFS from 2001-2005 using the Towed Optical Assessment Device (TOAD) and were analyzed according to the CRED classification scheme (www.soest.hawaii.edu/pibhmc) that includes factors such as substrate type, living growth on the seafloor, geomorphic zone, and the size and abundance of holes in the substrate.

Methods

Initially we took a qualitative approach to seafloor characterization by visually comparing the data. For example, we related backscatter intensity and seafloor slope to the substrate type, as determined by the optical data, by observing that hard (rock, rubble) and coral-rich substrate correspond to high backscatter intensity and slope, while soft (sandy) substrate correspond to lower backscatter intensity and slope.

However, coral-reef and fisheries managers generally have little experience in interpreting optical and acoustic datasets to form management and monitoring plans. They stated the need for integrated map products and in particular the NWHI Papahaʻnaumokuaʻkea Marine National Monument (NWHIMNM) managers requested maps showing areas of hard and soft seafloor substrate that could be used in the framework of a habitat-based, stratified random sampling design (Ault et al. 1999). To accomplish this goal we took an image processing approach using the ENVI software package commonly used to analyze satellite imagery by the remote sensing and planetary science communities.

Acoustic backscatter data are the most useful dataset available for FFS for identifying hard and soft seafloor. Initially a supervised classification technique, which clusters pixels into pre-defined classes, was applied to the data. Region of interest (ROI) classes were chosen by querying TOAD data based on photos that were classified as either ≥80% hard bottom or ≥80% soft bottom (Fig. 2).

A 2-class maximum likelihood supervised classification algorithm was run on ENVI software using the 2 ROI classes and a 3-band image containing backscatter and small- and large-scale bathymetric variance. Variance, derived from the bathymetric data, is the square of the standard deviation of a pre-defined number of neighboring pixel cells. Variance calculations were performed using ArcGIS Spatial Analyst tools. Small-scale variance, calculated for a 3x3 pixel cell neighborhood, is useful for defining topographic features such as sediment ripples or possible coral-rich regions, whereas the large-scale variance, calculated for a 5x5 neighborhood, is useful for defining seafloor ridges, pinnacles, and significant changes in slope. Variance proved to be a useful bathymetric derivative in previous efforts to map seafloor habitats by Dartnell and Gardner (2004) and including both the small- and large-scale variance in our analyses generally gave better results despite their visual similarity (Fig. 3). The acoustic data were resampled to a 5 m grid cell size prior to running the ENVI analyses. Additionally, the Reson 8101 and Kongsberg EM3002D datasets were processed separately due to their different frequency contents and the resulting classified images were combined during post-processing.

Initial results from the supervised classification were unsatisfactory because obvious artifacts around data gaps were apparent. There was also concern that the technique relied too heavily on the optical data. Uncertainties in camera sled positioning, absence or limited availability of optical data for many areas, and bias in the supervised class definitions made it necessary to investigate an alternative approach.

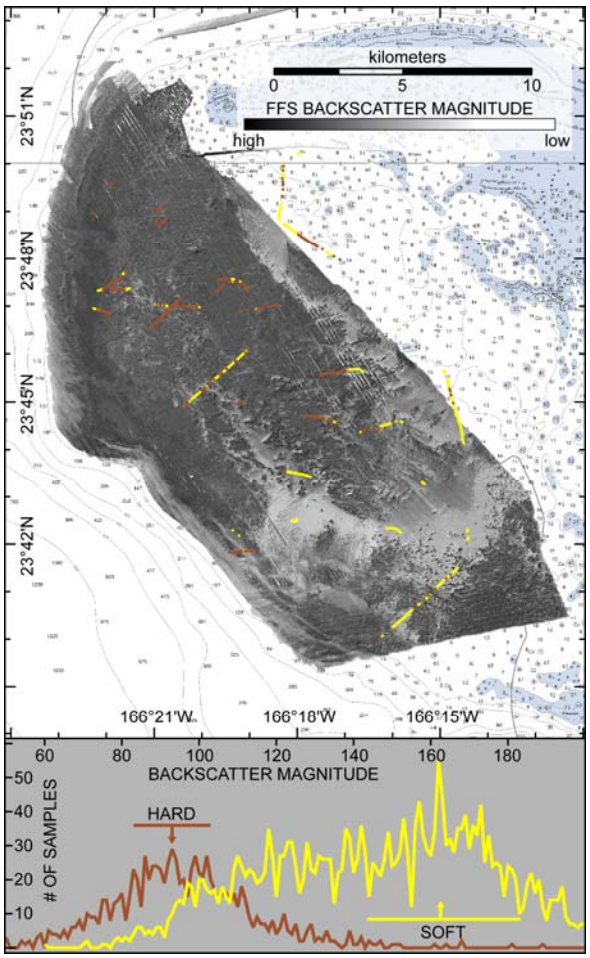

Figure 2: (Top) Backscatter imagery and TOAD tracks colored yellow for ≥80% sand/soft bottom and brown for ≥80% hard/rock bottom. (Bottom) Backscatter magnitude vs. number of samples for hard (brown) and soft (yellow) seafloor ROI's. Lower backscatter values are harder bottom due to the reversed polarity dataset used to extract the backscatter magnitude values for the plot. Vertical arrows and horizontal bars indicate the mean and standard deviation for each ROI respectively.

Consequently an unsupervised classification technique was chosen, which relies only on the data statistics. Using this technique, no user defined training classes or inputs other than the acoustic data are required for creating the classified image. After testing different combinations of input data and various classification algorithms a 2-class K-Means unsupervised classification algorithm was run on ENVI software using a 4-band image containing backscatter, bathymetric rugosity, and small- and large-scale bathymetric variance (Fig. 3) to derive regions of hard and soft substrate for the mapped portion of the bank top. Rugosity is calculated using the Benthic Terrain Modeler in ArcGIS (Jenness 2003). The unsupervised classification algorithm calculates class means evenly distributed in the data space and then iteratively clusters the pixels into the nearest class using a minimum distance technique. In general, the unsupervised classification dealt better with data gaps and delineation of isolated hard bottom areas surrounded by sand, such as pinnacles, than did the supervised technique. The hard and soft classes were assigned to the resulting unsupervised image by visually comparing the optical data with the classified image.

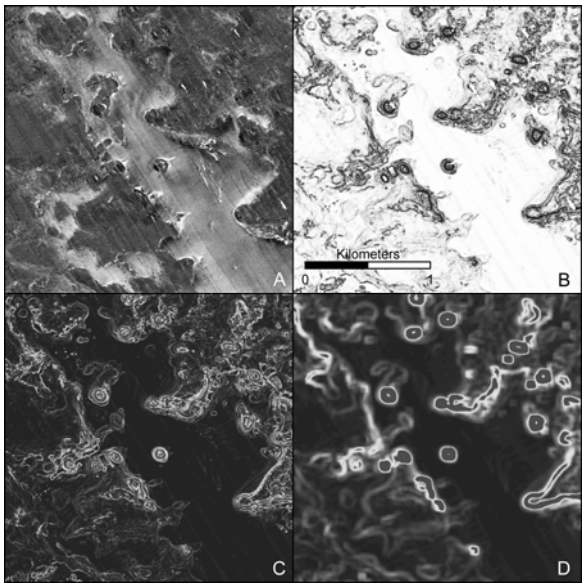

Figure 3: (A) Backscatter, (B) bathymetric rugosity, (C) small-scale bathymetric variance, and (D) large-scale bathymetric variance for a small subset of the FFS bank top. Note the 1-km-long scale bar in (B). Higher backscatter is indicated by darker colors in (A), higher rugosity is indicated by darker colors in (B), and higher variance is indicated by lighter colors in (C, D).

Results and Conclusions

Figure 4 shows the hard and soft seafloor substrate map created using the unsupervised classification technique for a portion of the bank top at FFS. A confusion matrix was calculated to compare the classification results with the ground truth (TOAD) data. The overall accuracy of the classification results when compared to the user-defined ROI classes is ~80% but the accuracy is much less (~30%) when the results are compared with the entire TOAD dataset. This difference is most likely associated with the positional uncertainties of the TOAD data and errors in the unsupervised classification and suggests that visual comparison of the classification results with the optical data is imperative for assigning substrate types to classes. Subsequent optical and SCUBA-based ground truth operations at FFS suggest the ~80% accuracy is a realistic overall assessment of the unsupervised classification results.

Substrate maps, produced as describe above, are currently being used by NWHIMNM scientists and managers to determine long term monitoring sites. It appears that this is an appropriate approach for

integrating high-resolution multibeam survey datasets covering large areas with limited coverage optical datasets. This method can easily be expanded if high quality acoustic and optical data are available, it has been applied to a number of other areas, and the resulting maps and data are available for download at www.soest.hawaii.edu/pibhmc. The processing approach described above and error assessment techniques continue to be refined to create accurate seafloor substrate maps.

CRED study sites span the tropical Pacific Ocean basin and data acquisition is often limited by the amount of time available to survey in remote locations. This can result in maps covering a wide area with sparse coverage and some data gaps. The NOAA Biogeography Branch is applying similar image processing techniques to create spatially limited seafloor substrate maps with dense coverage and few data gaps around islands in the Caribbean, such as Puerto Rico (Costa et al. 2008). However, the specific method described here has been developed to meet needs of resource managers in coral reef ecosystems with somewhat sparse multibeam and minimal optical data.

Although logistical constraints may limit the coverage of acoustic datasets (multibeam and backscatter), experience has demonstrated the importance of designing surveys to collect high quality backscatter data. Greater coverage can be attained by concentrating only on maximizing data collection. However, the slower survey speeds and other constraints that may be required to collect high quality backscatter data have been found to be justified because it is undoubtedly the best discriminator of hard and soft seafloor. Additionally, dense grids of optical ground truth data aid tremendously in class determination, derived layer validation, and error assessment.

The coral reef community has only begun to apply image processing techniques (e.g. Dartnell and Gardner 2004; Blondel and Sichi 2008; Marsh and Brown 2008) to acoustic and optical datasets for habitat classification and characterization. These methods represent emerging techniques with much broader characterization and predictive possibilities. For example, predictive habitat mapping (Pittman et al. 2007) expands the utility of acoustic and biologic datasets to predict biomass and fish species abundances based on marine organism seafloor habitat preferences.

Acknowledgement
We thank the NOAA Coral Reef Conservation Program for funding ongoing coral reef ecosystem research throughout the U.S. Pacific Islands. We also thank HMRG for help with data processing and interpretation.

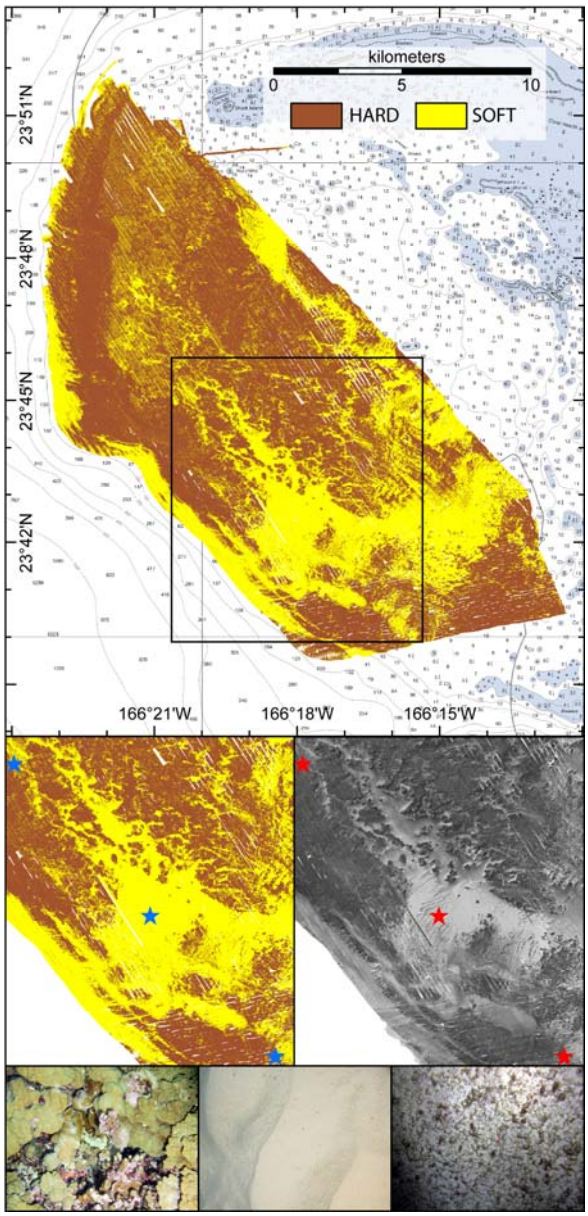

Figure 4: (Top) Hard and soft seafloor map created using the ENVI K-Means unsupervised classification algorithm on a 4-band input image. Black box corresponds to the area shown in the images beneath. (Middle) A portion of the hard and soft (left) and backscatter (right) data. Stars indicate the TOAD frame grab locations shown beneath. (Bottom) TOAD frame grabs that, from left to right, correspond to the star locations from top to bottom (northwest to southeast). Coral-rich (left), sandy (middle), and pavement (right) seafloor substrates match the hard and soft classes.

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Comparing Semi-Automated Methods for Classifying Multibeam SoNAR & Airborne LiDAR Imagery

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Abstract. Benthic habitat mapping supports ecosystem-based management objectives by contributing to the development of detailed species utilization models, which link physical habitats to biological information. Shallow to deep-water marine habitats have been successfully characterized by manually digitizing and attributing optical and acoustic remotely sensed imagery. These resulting maps, while extremely useful, are subjective and ultimately irreproducible because they depend on the accuracy and interpretation of the person that is digitizing. Here, we semi-automate the seafloor feature extraction and classification process of high-resolution multibeam (MBES) sound navigation and ranging (SoNAR) and light detection and ranging (LiDAR) imagery using three classification techniques—Support Vector Machines (SVM), Classification & Regression Trees (DTC) and Object-based Segmentation (OBS). The ability and accuracy of these three techniques to delineate and characterize seafloor features was qualitatively compared at relevant spatial and thematic scales using georeferenced underwater imagery. Overall, we found that DTC and OBS out-performed SVM, as these two methods produced habitat maps that were qualitatively more accurate. This analysis suggests that DTC and OBS have the potential to help scientists more quickly and objectively characterize the seafloor, allowing resource managers to better assess the changing health of coral reef ecosystems.

Key words: LiDAR, multibeam SoNAR, MBES, coral reef ecosystems, benthic habitat mapping

Introduction

Benthic habitat mapping supports ecosystem-based management objectives by contributing to the development of detailed species utilization models, which link physical habitats to biological information (Friedlander and Parrish 1998; Pittman et al. 2007). Shallow to deep-water marine habitats have been successfully characterized by manually digitizing and attributing optical and acoustic remotely sensed imagery (Kendall et al. 2005; Battista et al. 2006). These resulting maps, while extremely useful, are subjective and ultimately irreproducible because they depend on the accuracy and interpretation of the person that is digitizing.

In the following study, we address this problem of subjectivity by semi-automating the seafloor feature extraction and classification process of high-resolution MBES and LiDAR imagery using three separate classification techniques. These three techniques—SVM, DTC and OBS—are fundamentally different in how they partition an image into separate classes.

SVM separates an image into classes by maximizing the margin between two groups of pixels in *n*-dimensional space (Vapnik et al. 1997). This margin is delineated using two parallel hyperplanes. The points nearest to these hyperplanes are called support vectors.

DTC separates an image into classes using binary recursive partitioning (Breiman et al. 1984), which is an iterative process whereby image pixels are split into two groups that are as homogenous as possible. The process continues until no more useful splits are found.

OBS separates an image into classes using edge detection algorithms (Haralick 1979; Blaschke et al. 2000). These algorithms detect the boundaries of homogenous groups of pixels. The pixels within each group are similar to each other based on a combination of characteristics, such as intensity and texture.

Given the methodological differences, we qualitatively compared these techniques to determine which was the most accurate,

efficient and robust method for semi-automating the feature extraction and classification process. In doing so, we addressed the following key research questions:

(1) Which of these classification techniques most accurately delineated biologically & geomorphologically relevant habitats in Abrir La Sierra Conservation District?

(2) What are the strengths and weaknesses associated with each semi-automated classification technique?

Materials and Methods

Description of Study Site

The study site, Abrir La Sierra Conservation District, is a marine protected area (MPA) that is located 20 km off the western coast of Puerto Rico. This 16.7 km^2 region was designated as an MPA in 1996 due to concerns over declining reef fish populations (Waddell and Clark eds. 2008). The economically important species, red hind (*Epinephelus guttatus)*, was of particular concern because it uses Abrir La Sierra as a spawning aggregation site (SPAG). SPAGs are crucial to the ecology of an area because they help replenish fish populations, keeping their numbers relatively stable year after year. Since these aggregation sites are so vital, a concerted effort has been made to better understand them by mapping and monitoring changes in their associated benthic habitats.

Data Acquisition: System Specifications

Bathymetry and intensity imagery were acquired for the Abrir La Sierra using MBES and LiDAR. "Bathymetry" denotes the depth of the seafloor and was calculated by measuring the time required for an individual pulse of light or sound to travel from the sensor to the seafloor and back again. "Intensity" denotes the amount of light or sound that was scattered back to and recorded by the sensor. Data from both sensors were collected and processed to meet the International Hydrographic Organization Order 1 horizontal and vertical accuracy standards.

LiDAR data were acquired between 4/7 and 5/15/2006 for elevations between 19 m above sea level down to 50 m below sea level using a Laser Airborne Depth Sounder (LADS) Mk II Airborne System. This airborne system uses a 900 Hz neodymium-doped yttrium aluminum garnet laser, which is split by an optical coupler into an infrared (1064 nm) beam and a green (532 nm) beam. The infrared beam measures the datum height at nadir, and the green beam oscillates across-track to measure depths and/or elevations. The airborne survey was flown at altitudes between 1,200 and 2,200 ft. The raw data were logged using the Tenix LADS Airborne System and were converted using the LADS Mk II Ground System. The soundings were referenced to the NAD83 UTM 19 N horizontal coordinate system and to the Mean Lower Low Water (MLLW) vertical coordinate system (Stephenson and Sinclair 2006).

MBES data were acquired between 4/14 and 4/24/2007 for depths between 21 and 290 m using a Simrad 1002 (95 kHz) MBES. This hull-mounted, ship-board system has 111 electronically stabilized beams with 2° widths, rectilinearly arrayed 75° to the port and starboard of nadir. It achieves seafloor coverage of 7x water depth down to 1,000 m. The vessel's positioning and orientation were determined by the Applanix POS/MV 320 V4. The data parcels were logged using Kongsberg's MERLIN v5.2.2 acquisition and control program, and were referenced to the NAD83 UTM 19 N horizontal coordinate system and to the MLLW vertical coordinate system (Battista and Stecher 2007).

The LiDAR bathymetry and intensity surfaces were created at 4x4 and 5x5 m spatial resolutions (respectively) using CARIS Base Editor v2.0 software. The MBES bathymetry and intensity surfaces were processed and created at 5x5 and 1x1 m spatial resolutions (respectively) using CARIS Hips & SIPS v6.0 and Geocoder v3.2.1 software (Fonseca and Calder 2005). Both the LiDAR bathymetry and the MBES intensity surfaces were resampled to 5x5 m resolutions. All of the surfaces were exported as GeoTiffs and ingested into ArcGIS 9.2 for further analysis.

Ground Validation: Underwater Video Data

Georeferenced imagery of the seafloor was collected using NOAA Biogeography Branch's drop camera system between 4/14 and 4/24/2007. This system is equipped with lights, scaling lasers, an acoustic transponder, a digital video camera and a high resolution still camera (ccma.nos.noaa.gov/products/biogeography/usvi_nps/camera.html). The integration of these components allows for the collection of highly resolved and spatially accurate underwater imagery.

Each suitable, underwater image was visually interpreted and attributed by a trained observer using a hierarchical habitat classification scheme. This scheme defined broad and fine-scale benthic habitats on the basis of 13 geographic zones, 12 unique geomorphological structure types and 4 unique biological cover types using a variable minimum mapping unit (www8.nos.noaa.gov/bhv/bhvMapBrowser.aspx). The broad structure types included algal nodule pavement, bedrock, mud, pavement, sand and scattered coral and rock. The cover types included coral, macroalgae, turf and uncolonized. The resulting classified images were used to train the machine learning algorithms and to qualitatively validate the preliminary habitat classifications. A robust, quantitative accuracy assessment was not conducted because there were insufficient numbers of independent points (> 25) per unique thematic habitat class.

Data Processing: Surface Creation & Selection

A suite of metrics describing the complexity of the seafloor was created from the LiDAR and MBES bathymetric surfaces to aid in the identification and classification of benthic habitats. These morphometrics included: standard deviation of water depth, rugosity, slope, curvature & fractal dimensions (Table 1).

Each of these metrics were re-calculated at a range of spatial scales (10, 30, 50 & 75 m radii) using a median, circular moving window in ArcGIS 9.2. This multi-resolution approach was taken because fish and corals respond to benthic habitats at varying spatial scales (Kendal et al. 2003; Pittman et al. in press). These different metrics were then stacked in ENVI 4.4, creating a single composite image for each data type at each spatial scale (Fig. 1). The principal components of these composite images were calculated to determine the degree of correlation between pairs of metrics. The least correlated metrics were then included in the semi-automated classification.

Morphometric	Unit	Description	Analytical Tool
Standard deviation (SD) of water depth	Meters	Dispersion of water depth values about the mean	Focal statistic in ArcGIS Spatial Analyst
Rugosity	Ratio value	Ratio of surface area to planar area	Benthic Terrain Mapper toolbox (Jenness, 2002)
Slope	Degrees	Maximum rate of change in slope between cell and eight neighbors	ArcGIS Spatial Analyst's slope function
Curvature	1/100 z units	Rate of change in curvature across the surface highlighting ridges, crests and valleys	Curvature function in ArcGIS 3D Analyst
Fractal dimension (*D*)	Unitless	A measure of surface roughness with values between 2 and 3	FocalD script in LandSerf 2.2 (Wood, 2005)

Table 1. Description of morphometrics used to characterize and classify the homogenous habitats on the seafloor.

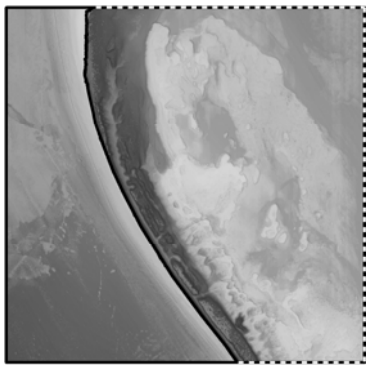

Figure 1. These composite images depict intensity, depth and slope surfaces as individual RGB bands. The imagery outlined by the black line (left) was acquired using MBES. The imagery outlined by the dashed line (right) was acquired using LiDAR.

Comparing Classification Methods

Several steps were needed to classify these composite images. The first step was to create regions of interest (ROIs) using the classified, video imagery. These ROIs were used to train each of the machine learning algorithms, which were subsequently deployed in ENVI original and ENVI Zoom v4.4. After each algorithm was run initially, the resulting outputs were refined by incrementally adjusting key input parameters. It was, however, difficult to objectively determine if these parameters were

optimized without being able to conduct a quantitative accuracy assessment. Such an assessment should be conducted in the future, allowing for the explicit optimization of these parameters.

The final step in this process was to visually compare the outputs from the three different methods to determine their qualitative accuracy. Surfaces with "high" qualitative accuracy: (1) did not confuse one habitat type with another; (2) did not identify geometric or radiometric artifacts in imagery as habitat features; (3) were capable of detecting subtle spectral differences between habitat types in the imagery; and (4) were capable of concurrently delineating fine and coarse-scale habitat features (e.g., individual patch reefs and aggregate patch reefs). Surfaces with lower qualitative accuracy did not meet one or all of these criteria.

Results

Classification Accuracy of LiDAR Imagery

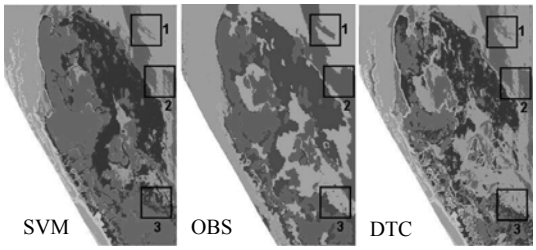

Figure 2. The final classified surfaces developed from the LiDAR imagery using SVM (left), OBS (middle) and DTC (right). Areas 1, 2 and 3 denote locations where the classified results did not meet one or several of the qualitative accuracy criteria as outlined above.

The three different machine learning techniques produced noticeably different classification results when applied to the LiDAR imagery. Overall, DTC produced (qualitatively) the most accurate result of the three methods. SVM and OBS produced less accurate results for two primary reasons: (1) they consistently confused one habitat class (sand) with another habitat class (low rugosity pavement) (Fig. 2, Area 1); and (2) they did not identify and extract many fine-scale features (reef holes) in the imagery (Fig. 2, Area 3). Despite DTC having produced a more accurate result, OBS was more resilient to geometric or radiometric artifacts in the imagery, and was less likely to identify them as habitat features (Fig. 2, Area 2).

Classification Accuracy of MBES Imagery

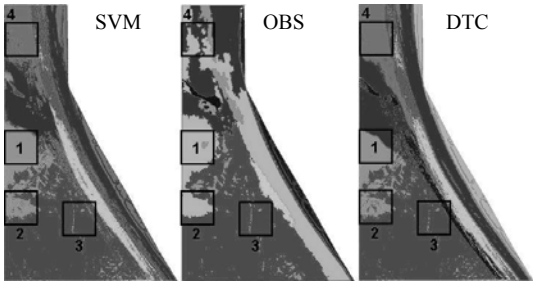

Figure 3. The final classified surfaces developed from the MBES imagery using SVM (left), OBS (middle) and DTC (right). Areas 1, 2, 3 and 4 denote locations where the classified results did not meet one or several of the qualitative accuracy criteria as outlined above.

The three different machine learning techniques also produced noticeably different classification results when applied to the MBES imagery. Overall, OBS produced qualitatively the most accurate result out of the three methods. SVM and DTC produced less accurate results for three primary reasons: (1) they consistently confused one habitat class (i.e., pavement) with another habitat class (i.e., bedrock) (Fig. 3, Area 1); (2) they consistently identified geometric or radiometric artifacts in the imagery as bona fide habitat features (Fig. 3, Area 2); and (3) they failed to detect subtle spectral differences between habitat types in the imagery (Fig. 3, Area 4). All three methods adequately extracted both fine and coarse-scale features simultaneously (Fig. 3, Area 3).

Discussion

A qualitative analysis of the three semi-automated techniques and their final, classified outputs revealed that each technique had specific strengths and weaknesses. These strengths and weaknesses were most likely the combined result of the algorithm's input parameters, the different spatial resolutions of the MBES and LiDAR surfaces, the shape of the re-scaling kernel, and the correlation between surface metrics. Changes to any of these parameters could have altered the classification results.

That being said, overall, DTC outperformed the other methods when applied to imagery with spectrally distinct habitats, and to imagery with few geometric or radiometric artifacts. It was also the fastest of the three methods, as it took the least amount of time to produce an

accurate, classified image. OBS, on the other hand, was the slowest of the three techniques, albeit it did outperform the other methods when applied to imagery that had geometric or radiometric artifacts and to imagery that had habitats with similar spectral signatures. Overall, DTC and OBS both outperformed SVM because their surfaces had higher qualitative accuracies. SVM was most likely outperformed because it had many more parameters to adjust and optimize than did the two other methods.

A final benthic habitat map was created using the DTC surface developed from the LiDAR imagery and the OBS surface developed from the MBES imagery (Fig. 4). This seamless map characterized 18 unique seafloor habitats in 10 to 290 m of water.

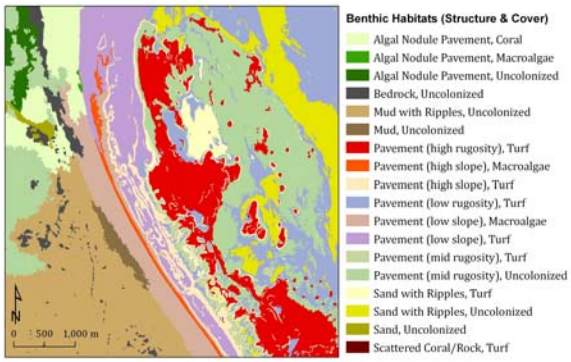

Figure 4. This graphic depicts the final, seamless benthic habitat map of Abrir La Sierra Conservation District. It was created by merging and edge matching the best classification outputs developed from the LiDAR and MBES imagery.

In conclusion, our current data and analysis indicate that DTC and OBS have the potential to help scientists create benthic habitat maps more quickly and efficiently. Future analysis (and a quantitative accuracy assessment) will improve this process further by objectively determining the optimal algorithm parameters. Hybridizing the OBS and DTC methods may also contribute to this improvement by integrating of the strengths of both techniques. The accuracy, objectivity and repeatability of habitat mapping will allow resource managers to more frequently and reliably assess the changing distribution (and ultimately, health) of the coral reef systems that they manage. Improving our understanding of these ecosystems is the key to identifying and mitigating the heterogeneous threats that face these important and precious resources.

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Assessing the distribution of patch reef morphologies in the Lower Florida Keys, USA, using IKONOS satellite imagery

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Abstract. As live coral cover continues to decline in the Florida Keys, it becomes increasingly important not only to determine the location and abundance of live coral remaining, but also to understand why certain areas possess higher coral cover than others. At present, coral cover tends to be highest at shallow inshore patch reefs. Our study has two objectives: 1) to determine, to the full extent visible by satellite imagery, the number and characteristics of patch reefs that could be recognized using IKONOS imagery; and 2) to test the assumption that various morphological groups of patch reefs occupy distinct cross-shelf zones in the Lower Keys. Two previous survey efforts using aerial imagery and reported 420 and 750 patch reefs, respectively, from Big Pine to the Marquesas Keys. By performing a visual assessment on IKONOS satellite imagery, we were able to delineate 2,251 patch reefs for this region. These patch reefs vary in their overall morphology (i.e., shape) and are spatially distributed in several cross-shelf bands. Patch reef classes identified were Aggregate, Atom, Colony, Crescent and Dome. Aggregate patch reefs are very numerous, relatively small, and dominantly located either shallow-midshelf or offshore. Dome, Colony and Crescent patch reefs are larger in area and are most common in the shallow-midshelf or offshore zones. This study represents an important first step in understanding the factors that may be controlling the distribution and shape of patch reefs along the Florida Keys Reef Tract and, subsequently, relating this to living coral cover on modern reefs.

Key words: Florida Keys, patch reef, morphology, coral, remote sensing.

Introduction

Declines in overall live coral cover throughout the Florida Keys are well documented (Porter et al. 2002). The Florida Keys Coral Reef Evaluation and Monitoring Project (CREMP) has monitored 40 reef sites throughout the Keys since 1996. In 2005, coral cover on patch reefs averaged 14.6%, compared to 3.0% for the offshore bank reefs (Beaver et al. 2006). Coral cover has continued to decline on the offshore bank reefs so that live coral assemblages are now concentrated on shallow-water patch reefs. Thus, to adequately assess and protect remaining coral assemblages, it is essential to know the number, extent and spatial characteristics of the many patch reefs spread throughout the region.

The Florida Keys have historically been separated into three geographic regions: Upper, Middle and Lower Keys (Ogden et al. 1994). Previous efforts to determine the number of patch reefs in the Lower Keys utilized aerial survey methods. Marszalek et al. (1977) identified 420 reef structures from Big Pine to the Marquesas Keys. The Florida Marine Research Institute's Benthic Habitat Map of the Florida Keys estimated 750 patch reefs (inclusive of aggregate patch reefs) in the Lower Keys region (FMRI 2000).

Our study area encompasses a 523 km^2 region stretching from Big Pine Key in the northeast to Sand Key Reef in the southwest, and extends from the land to the outer reef system (depth≈20m) (Fig. 1). We used high spatial resolution, georeferenced IKONOS satellite imagery provided by the National Oceanic and Atmospheric Administration (NOAA) to find, categorize and map the size, shape and distribution of patch reefs. Initial observations indicated that patch reef distribution is non-random. Patch reefs appear to occur in distinct bands that parallel land, each with increasing distance from shore. There also appears to be segregation between patch reef morphologies (i.e., shape), with different types rarely sharing a given zone. The goals of this study were to 1) determine the number and characteristics of patch reefs that could be visually identified using IKONOS imagery and 2) test the assumption that various morphological groups of patch reefs occupy distinct cross-shelf zones in the Lower Keys.

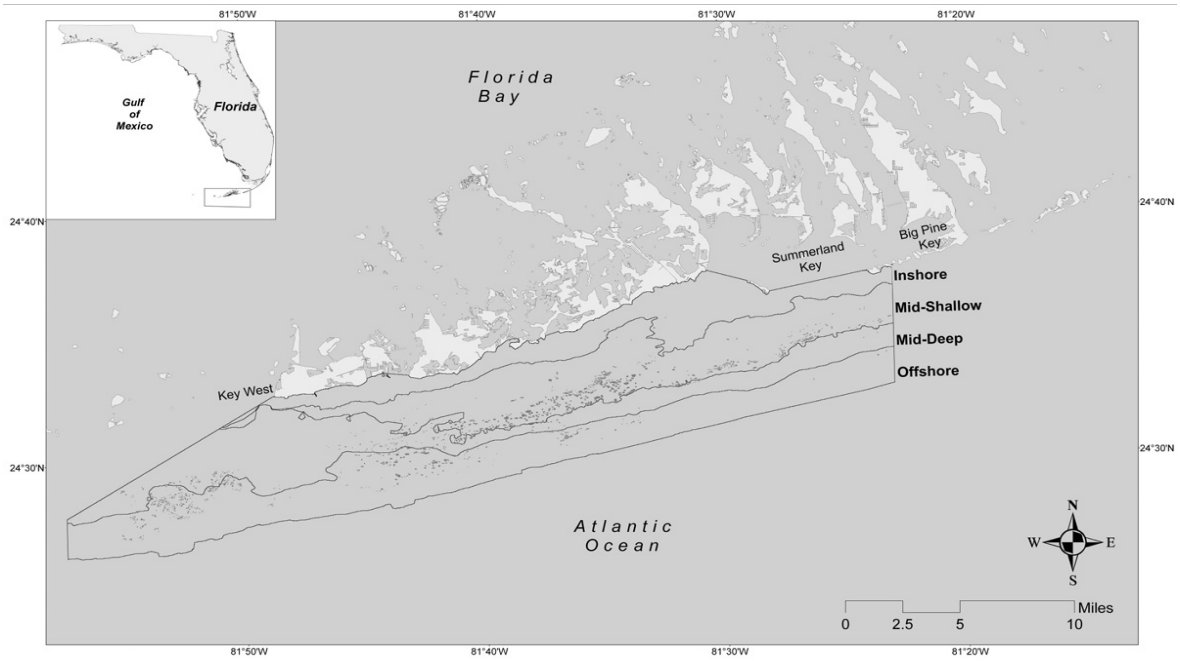

Figure 1: Lower Florida Keys study region displaying cross-shelf zones and patch reef distribution.

Methods

Data Source

Twenty-five IKONOS images acquired during 2006 and corresponding to the Lower Keys region were provided by NOAA. Of these, the four clearest images that provided complete coverage of the study region were selected for analysis, while the remaining 21 images were used to assist in later verification. IKONOS is a privately owned satellite (GeoEye) with a spatial resolution of 4 m that collects data in three visible wavelength bands (450 – 520 nm, 510 – 600 nm and 630 – 700 nm). The Lower Keys were segmented into four zones (Inshore, Mid-Shallow, Mid-Deep and Offshore) using digital bathymetry and clearly visible cross-shelf habitat breaks (Fig. 1). Digital bathymetry was compiled from three sources; NOAA Geophysical Data System (GEODAS), the Benthic Habitat map of the Florida Keys and an internal FWRI bathymetric dataset (Palandro et al. 2008).

Patch Reef Mapping/ Typology

The location of each patch reef was determined by visually examining each image. Cosmetic stretching was applied to the red, green, and blue bands (RGB) to enhance differences between suspected patch reefs and other benthic habitats. When a patch reef was found, it was marked as a point using ENVI 4.3 ® remote sensing software. The decision to classify a structure in the imagery as a patch reef was based on the appearance of a reef-like structure, surrounding benthic attributes (e.g., halo) and local knowledge.

The shape of each patch reef was delineated from the satellite imagery (Fig. 2) using all available overlapping imagery for a given area. The two to three clearest images (e.g. cloud-free, transparent water) were used to verify the presence of a patch reef. After the initial mapping of all visible patch reefs, the designation of each was further confirmed using ancillary datasets. These included satellite imagery, analog aerial imagery and interferometric acoustic data, as well as consulting with people familiar with the area. Final quality control measures for the dataset are still underway; therefore, for the purpose of this study, it is assumed that all reef structures identified in the imagery are in fact patch reefs.

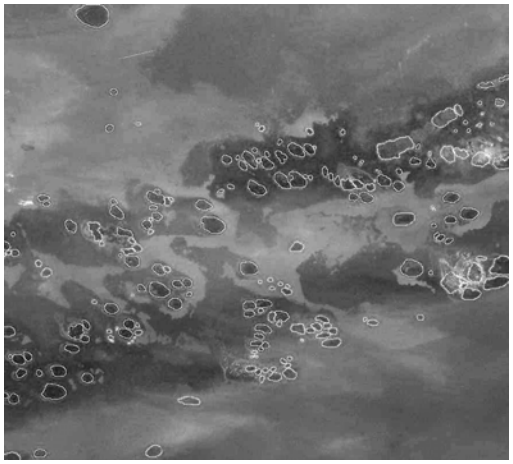

Figure 2: Examples of patch reefs delineated from IKONOS imagery. White versus gray outlines were used to differentiate between reefs during quality control analyses.

After determining the point location of each patch reef, a polygon was created of the entire reef area; the area contained within, and excluding, a visible sand halo. A sand halo is an area that generally fringes a patch reef and is created by grazing herbivores (Randall 1965, Ogden et al. 1973). The bright reflectance of the sand halo is in contrast to the darker return of the actual patch reef (contained within it) and provides a clear boundary for delineating only the reef area. The polygons that were created were exported to ArcGIS 9.3. Patch reef morphology in the Florida Keys has historically been divided into just two categories: dome and linear (Marszalek et al. 1977, Chiappone 1996). Patch reef morphology were classified here into a greater number of morphologies: Aggregate, Atom, Colony, Crescent and Dome (Fig. 3). Each of these morphologies can be considered a subset of the original 'dome' classification.

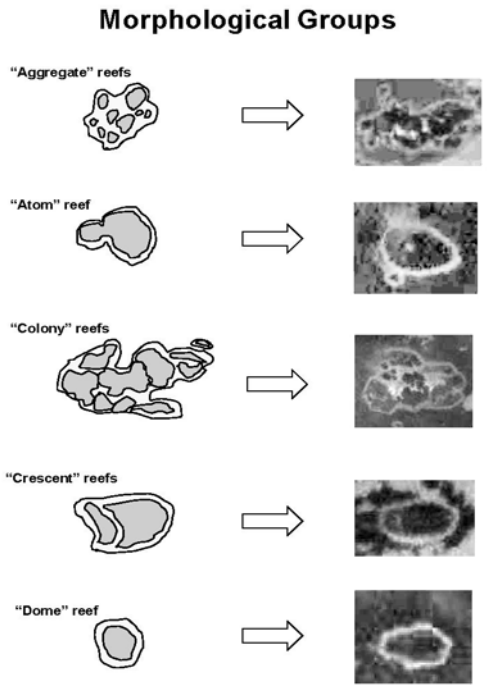

Figure 3: Categories of patch reefs identified.

Defining characteristics of each patch reef morphology are as follows:

Aggregate: a grouping of patch reefs of various sizes - generally three to ten in number – that share a common halo and exhibit complete separation between individual reefs.

Atom: a circular or elliptical-shaped patch reef containing a second patch reef within its sand halo. The secondary patch reef is always many times smaller in scale and is contained completely within the halo of the 'parent' reef, such that it appears in the process of budding off.

Colony: like Aggregate patch reefs, Colony reefs are found in close proximity to one another and share a common halo. Unlike Aggregate reefs they exhibit a 'conjoined' structure, for which a visible sand halo does not entirely separate individual reefs within the group.

Crescent: two patch reefs that share a single halo surrounding their exterior, and have complete separation in between via a sand channel. Crescent reefs always orient themselves in a northeast-southwest direction with the larger of the two reefs shaped like a 'bullet' with the smaller, 'boomerang-shaped' reef trailing in its wake.

Dome: the 'classic' dome patch reef; a single circular or ellipsoid reef with a single halo.

Patch Reef Metrics

Area and perimeter measurements (m^2 and m respectively) were obtained using XTools Pro for ArcGIS 9.3. The major axis of each patch reef was determined by placing a minimum area bounding rectangle around the delineated patch reef polygon. The major (long) axis of the rectangle then served as an estimate of the maximum diameter of the patch reef.

The complexity of each patch was defined by its perimeter to area ratio (P/A). Because this measure is sensitive to the size of the patch reef, a corrected perimeter to area ratio (PAC) was used that is not sensitive to changes in size of a patch of given shape. PAC index values range from 1.0 for a circle, 1.1 for a square, and can become infinitely large for long, narrow, and highly convoluted shapes (Baker and Cai 1992). PAC is defined as,

$$PAC = P / \sqrt{(4\pi \times A)},$$

where P is the perimeter of the individual patch reef polygon and A is the area contained within the sand halo. The PAC index was calculated for each polygon individually and then averaged across the morphological group.

The coastline of the Lower Keys was delineated as a polyline in ENVI and exported into ArcGIS. Distance from shore was derived as a linear measurement between the centroid of each patch reef polygon and the nearest point on the shore. The extreme south-western end of the shoreline defined by this study was Key West. Therefore, the shoreline value used for the patch reefs just inshore of Sand Key Reef would have been Key West. However, the distance from shore measurement for patch reefs west of Key West is greater than if the shoreline had been artificially extended westward toward the Marquesas. For our study, we used only the current land-sea boundary extending from the northern tip of Big Pine Key, to the western end of Key West, as our shoreline.

Table 1: Total number (N), Percent of Each Morphology by Number, Total Area (km^2), Percent of Each Morphology by Area, μ Area (m^2) and Area σ, μ PAC, μ Maximum Diameter (m) and Maximum Diameter σ (m), μ Distance to Shore (km) and Distance to Shore σ (km) for all patch reefs by morphology.

.

	N	Percent of Total N	Total Area (km^2)	Percent of Total Area	μ Area (m^2)	Area σ	μ PAC	μ Max. Diam (m)	Max. Diam σ (m)	μ dist. to shore (km)	dist. to shore σ (km)
Aggregate	1185	52.6	1.914	32.4	1614	1802	1.269	48.5	28.2	7.394	2.661
Atom	100	4.4	0.372	6.3	3719	4675	1.283	70.8	51.1	5.437	1.002
Colony	184	8.2	0.944	15.9	5132	8467	1.357	87.2	73.7	5.126	1.134
Crescent	93	4.1	0.673	11.4	7336	6812	1.301	107.2	51.7	4.917	0.772
Dome	689	30.6	2.005	33.9	2909	3538	1.259	64.2	38.3	6.014	2.407
Total	**2251**		**5.908**								

Results

The total number of patch reefs detected in the Lower Keys region was 2,251. Of these, the Aggregate morphology was the most numerous (1185) and was located the farthest from shore, averaging 7.4 km. The largest area of patch reefs was in Dome reefs, because of their large individual size and abundance (689). Dome reefs, along with Aggregate, Colony and Crescent patch reefs were abundant in the Mid-Shallow region (Fig. 4). Aggregate and Dome reefs comprise most of the patch-reef area on the outer shelf. Aggregate reefs tended to be much smaller (on average 1614 m^2) than the Dome reefs (2909 m^2), but because of their large numbers (1185 Aggregate patch reefs or 52.6% of the total number), the Aggregate patch reefs nevertheless represented the second largest group in terms of overall area (1.914 km^2 of the total 5.908 km^2, or 32.4% of the total area)(Table 1).

The distribution of all types of patch reef morphologies was heavily concentrated in the mid-shallow and offshore regions. This can be seen on the map in Figure 1, and is detailed in Figure 4.

The PAC shape index of each individual reef further reflects reef morphology. The corrected perimeter to area ratio (PAC) can serve as a useful indicator of the complexity of the reef shape, with a high PAC ratio indicative of a complex or elongated shape and a low PAC ratio indicating a compact, circular shape.

The mean PAC did not indicate substantial shape differences. The Colony reef shapes were the most complex, with PAC averaging 1.36. The Dome reefs were the least complex, and have PAC values averaging 1.26 (Table 1). As a whole, the mean PAC values were very similar among all of the morphologies.

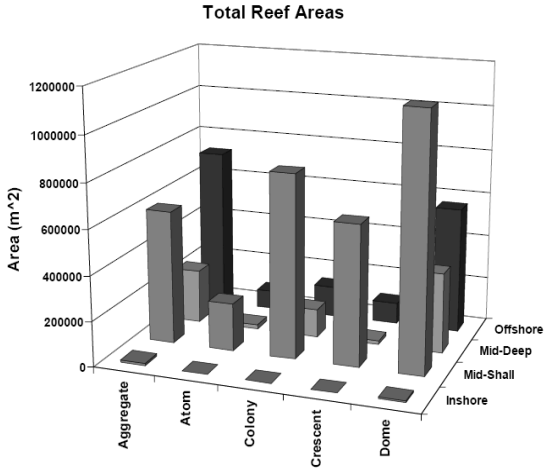

Figure 4: Total reef areas by class and distance from shore.

Discussion

The primary goal of this study was to identify and enumerate patch reef structures in the Lower Florida Keys Reef Tract using IKONOS satellite imagery. Using this method, 2,251 structures were identified. This is a substantial increase over previous estimates, more than six times Marzalek et al. (1977) and almost four times the FMRI (2000) estimates from aerial photography.

The qualitative characteristics that were initially observed in the patch reef dataset were not quantitatively demonstrated through the metrics we used. The patch reef data in Table 1 does not indicate that patch reefs have distinct morphologies and distributions in the Lower Keys, as was initially proposed. The summary data in Table 1 indicates that there exists high variability within morphological classes, and relatively small differences between them.

Future analyses will determine whether other metrics are more sensitive to – and can be used to quantitatively describe – the differences among patch reef morphologies

One inherent problem was that the reef parameters; area, perimeter, diameter, and the corrected perimeter to area ratio (PAC), were all affected by the 4 m pixel resolution of the original IKONOS imagery (i.e., polygons could not be delineated through the center of a pixel even if, in actuality, the edge of the reef may have). One possible way to better replicate the natural reef edge would be to use higher resolution (i.e. pan-sharpened or 1 m) imagery. This would alleviate this concern to a degree, however any delineation based on pixilated imagery would nevertheless result in a non-natural edge. A disadvantage of using higher resolution imagery is that it would greatly increase the amount of time spent analyzing the images.

A second possible limitation was that the patch reefs were subjectively classified by the observer and the data on a given reef were categorized under that class. Future multivariate statistical analyses will examine the reef-associated parameters independent of previously assigned classes.

Previous studies cited Florida Bay water and mobile calcareous sands as primary determinants of patch-reef growth, with patch-reef development focused on trough edges bordering Hawk Channel (Lidz et al. 2006). Key to our long-term analysis will be to determine how environmental and geological processes influence patch morphology and how this is manifested in the spatial distribution of patch reefs.

As live coral cover continues to decline, the importance of patch reefs in the Florida Keys will continue to increase. Under the working assumption that these patch reefs possess greater live coral cover than the offshore bank reefs, a comprehensive knowledge of their abundance, distribution, and controlling ecological parameters will be paramount.

Acknowledgement

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Macroalgal distribution in a Mexican Caribbean Reef

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Abstract. Macroalgae are important components of coral reef and seagrass ecosystems; however, the habitat structure macroalgae provide to the system has been little explored. We use architectural and morphological traits of macroalgae to describe and compare the reef and lagoon habitat complexity at Puerto Morelos reef in the Mexican Caribbean. Using a random-stratified method, macroalgae were sampled, then each organism was identified and morphometric features were recorded. A total of 101 species were found. The ordination analysis showed differences in species composition between the reef and lagoon. Small size, erect highly branched and dense clonal growth were more frequent in the reef compared to the lagoon where larger, erect, less branched and less dense algae with CaCO3 were found. Higher species richness and diversity of forms in the reef suggest a higher habitat complexity provided by macroalgae in the reef compared with the seagrass beds. The increase of fleshy macroalgae has been shown to decrease the habitat complexity provided by coral structure, we highlight that the increase of only some macroalgae species will result in a loss of complexity provided by other macroalgae species.

Key words: Plant architecture, clonal growth, Coral reef, Mexican Caribbean, Plasticity, Shift-phase

Introduction

Macroalgae are important components of coral reef and seagrass ecosystems, they help cement the reef framework and give refuge for numerous invertebrate and fish species (Gil et al. 2006); however macroalgae are increasing in abundance as a synergistic response to decrease of herbivores, increase of nutrients inputs, coral bleaching and coastal development, in both coral reefs and seagrass environments (Hughes et al 2005, Orth et al. 2006, Collado-Vides et al. 2007). Therefore a better understanding of the different roles macroalgae play on reef and seagrass systems is needed in order to protect and manage these resources.

Spatial complexity has been recognized as an important characteristic of habitats and is a major determinant of local abundance and diversity (Dahl 1973, Gratwicke and Speight 2005); moreover, architecture and density of macroalgae can influence the relationship between abundance and recruitment of other species in the community (Carr and Hixon 1995), therefore adding complexity to the reef system. For example, Hacker and Steneck (1990) demonstrated that architectural elements of algae (space between fronds) can be an important factor in determining demographic patterns of amphipods. Interpretation of macroalgal communities in marine habitats has been facilitated by the classification of species into functional-form groups proposed by Littler and Littler (1980), and Steneck and Dethier (1994). This approach characterizes species according to features such as body plan, behavior and/or life history strategy. Based on fundamentals from these authors we suggest that different habitats will be inhabited by different functional form groups, moreover, we suggest that these groups will in turn express different levels of habitat complexity.

The Puerto Morelos Reef system, in the Mexican Caribbean, is in a relatively good condition, and has a rich macroalgal flora both in corals as well as seagrass beds (Collado-Vides et al. 1998). We selected this site to explore a combination of community aspects such as: macroalgae community structure, functional form group distribution, and several architectural parameters as proxies to the evaluation of habitat complexity. In this study we describe and compare the reef and lagoon, adding a spatial structure perspective to the macroalgal community.

Material and Methods

This study was carried out at the Puerto Morelos reef, a section of the Mesoamerican reef system in 1998. The reef profile in Puerto Morelos has been divided into four main zones —lagoon, back-reef, crest, and fore-reef zone— based on its topographic

characteristics, coral species composition, and wave action (Jordan 1979). Substrate, water movement, and light are the most contrasting physical factors differentiating the lagoon and the reef area. The coral reef formation consists of corals of the genera *Porites*, *Acropora* and *Agaricia*, and some areas intermix with soft coral. The reef lagoon is characterized by seagrass meadows of the species *Thalassia testudinium* Banks ex Koenig mixed with bare sand patches and S*yringodium filiforme* Kuetzing in Hohenacker. Nutrient sources come mainly from the mangrove watershed through underground systems opening in springs in the reef lagoon.

Along this system, three contrasting areas were chosen as representatives of the range of habitat types: A) the fore reef and crest; B) deep lagoon and C) shallow lagoon. An imaginary line was laid out parallel to the shore trough these habitat types and these ones were considered as replicate transects. Each transect was then divided in five points: 1) La Bocana; 2) Astilleros; 3) ICMyL; 4) La Ceiba and 5) El Pueblo (Fig. 1). At each point, macroalgae were collected from four, non-overlapping, randomly-placed quadrants (0.25 m^2), for a total sample area of 5m^2 for each habitat. Based on the findings of Collado-Vides et al (1998), this sample area accounts for the maximum species found at each habitat type. All complete algal thalli present in the quadrants were collected by hand by SCUBA divers. Material was identified at species level and classified accordingly to morphological groups suggested by Steneck and Dethier (1994). In addition the following morphometric parameters were recorded in each plant: size (length in cm), type of growth (clonal or solitary); branching or frond density (non-medium-high); gregarious habit (solitary-regular-compact), attachment system (holdfast, rhizoids, stolon), anatomy (i.e. filament, blade, medullar) and presence-absence of $CaCO_3$.

Distributional patterns of species, in the range of habitat types, were analyzed by means of multivariate techniques that were applied to the presence-absence data matrix. A Bray-Curtis similarity matrix was obtained. No previous transformations were required. An ordination analysis by means of a non-metric multidimensional scaling program (MDS) was carried out. The Kruskal stress coefficient was applied in order to test the ordination obtained by the MDS using PRIMER VI package (Clarke 1993). A two way ANOSIM was used in order to test for significant differences between environments and seasons.

A contingency table was created with the distribution of species based on form-functional group classification. An X^2 was applied to the contingency table in order to find if the distribution of form-function groups were randomly distributed or differ as their interaction with environment. An ANOVA test was applied to the morphometric data comparing reef, lagoon and species present in both places. All statistic tests were conducted using the statistical package SPSS V. 13.

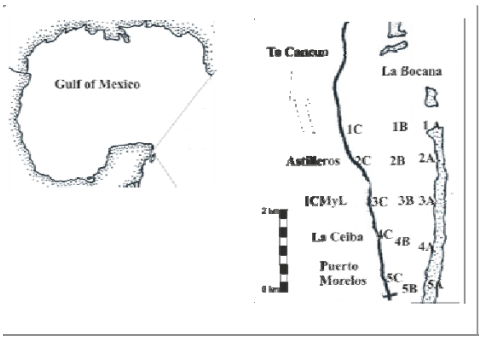

Figure 1: Study site and sampling stations. A= Reef sites, B= deep lagoon sites, C= shallow lagoon sties.

Results

A total of 101 species were found with a clear floristic difference between the reef and lagoon. A higher number of species, diversity and forms in the reef compared with the lagoon were found. Rhodophyta, which generally need hard substratum for attachment, were dominant in the reef, while Chlorophyta with sand-dwelling holdfasts were dominant in the lagoon. The most frequent large species found in the shallow and deep lagoon were the drifting brown species *Lobophora variegata* and *Dictyota bartayresiana* and sand-welling green species *Halimeda* spp., *Udotea* spp. and *Penicillus* spp; and no differences were found among seasons (Fig 2).

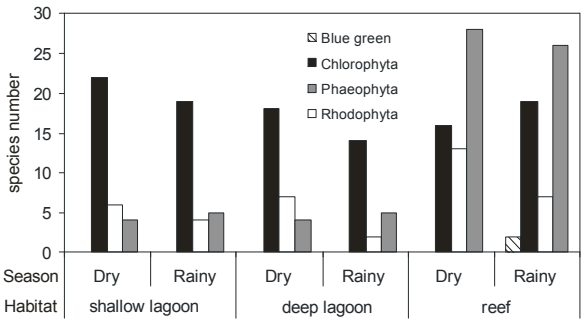

Figure 2. Total number of species at the reef, shallow and deep lagoon during both seasons sampled.

Species richness showed significant differences by habitat ($p<0.01$), with the highest richness found in the reef compared with the shallow lagoon and deep lagoon (23.1 ± 3.5, reef, *vs* 13.4 ± 3.9 and 12.9 ± 2.6, shallow and deep lagoon respectively). The MDS ordination analysis (Fig. 3) showed different species composition for the reef compared with the lagoon,

however shallow and deep lagoon were very similar among themselves (ANOSIM p>0.05).

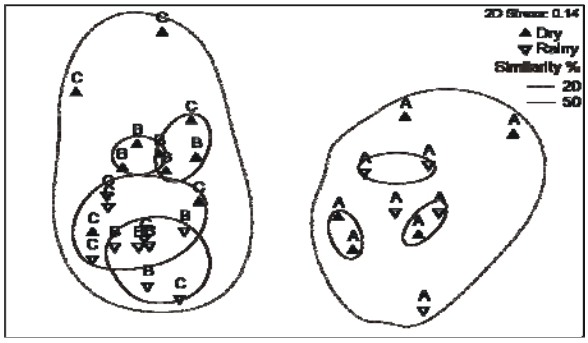

Figure 3: MDS configuration of species similarity matrix (stress= 0.14) for both seasons sampled and all sites. A= Reef, B= Deep lagoon, C= Shallow lagoon. Different line pattern shows different level of similarity between sites of different habitats.

The distribution of species classified on form-functional groups, demonstrated that different groups were present in the reef compared to the lagoon, x^2 = 63.37 (F=4, P= 0.0001) therefore form-functional groups were not randomly distributed. A higher diversity of forms was found in the reef with all 8 form-functional groups present, with the highest number of species belonging to the group of filamentous (turf forming) and corticated macrophytes. Five groups were present in the lagoon with the highest number of species belonging to the coenocytic group. Species present in both environments belonged to five form-functional groups without any group being dominant above the others (Table 1).

Table 1. Number of species belonging to each functional group and its frequency by habitat A= Reef, BC= Lagoon (shallow and deep), D= Reef and Lagoon. 1= Filamentous algae (uniseriate), 2 = Foliose algae (single layer), 2.5 = Corticated Foliose algae, 3 = Corticated macrophytes, 4 = Leathery macrophytes, 5 = Articulated calcareous, 6 = Coenocytic algae, 7 = Globular algae.

	Habitat			
FFG	**A**	**BC**	**D**	**Total**
1	20	0	3	23
2	2	0	0	2
2.5	5	1	7	13
3	17	1	4	22
4	6	0	0	6
5	9	3	5	17
6	6	18	5	29
7	6	1	0	7
Total	71	24	24	119

The morphological and architectural parameters had a distinct distribution of features among both environments. Small short organisms were found in the reef compared with those of the lagoon. Deep and shallow combined (ANOVA p<0.013), however no differences were found between reef and lagoon species that were present in both places (p<0.264), species present in both environments tended to have medium values, smaller than in the lagoon, but larger than the reef.

The orientation of the thallus: erect vs. prostrate reflects the way the thallus colonizes space, 85% of the reef and 87% of lagoon species had erect fronds with no clear stolon above ground, however all species that were found in both environments had prostrate arrangement of the thallus, mainly *Caulerpa* species. The attachment structures of the reef species were holdfasts or rhizoidal elongations which are strongly attached to hard substratum. Lagoon species had mainly rhizoidal systems either bulbous or just rhizoidal elongations that were embedded in the sand. Branching and gregarious habit which reflects how the thallus are packed in space, were significantly higher (p<0.04) in the reef compared to the lagoon showing a more compact and dense growth in the reef than in the lagoon. Species found in both environments tended to be more or less solitary with medium values of branching, among both extremes. More species with $CaCO_3$ were present in the lagoon than in the reef.

Discussion

The species composition found was similar to the species previous reported for the studied area and region (Collado-Vides et al 1998 for a review), at least in 1998 no strong shifts or macroalgal blooms were detected in the area, therefore this description can be used as a macroalgae base-line for present and future studies in Puerto Morelos, as no published historical records were found.

Species richness was higher in the reef than in the shallow and deep lagoon, this is consistent with the suggestion of McCoy and Bell (1991) that structural complexity of habitats is a major determinant of local abundance and diversity. The higher macroalgae species and architectural diversity found in the reef compared with the lagoon could be explained by the fact that corals offer a more complex environment due to the heterogeneity inherent to the form and shape of hard corals. This heterogeneity results in differences, at small scale in available space, water movement, and irradiation; therefore provide a greater spectrum of resources for macroalgae than seagrass beds (MacArthur and Levins 1967, Dahl 1973, McCoy & Bell 1991, Gratwicke and Speight 2005, Toohey et al. 2007).

Complexity in reefs is usually associated with different species of corals (Beukers and Jones 1997). Our study demonstrated that at least seven different

algal form-functional groups are present in the reef, adding layers of complexity to the already heterogeneous reef system. This is relevant as dominance of groups such as filamentous uniseriate algae, foliose algae (*Dictyota* type), and articulated calcareous green algae (*Halimeda* type), have been reported as main problems in reef systems going trough phase shifts due to environmental changes, (Gardner et al. 2003, Orth et al. 2006, Collado-Vides et al. 2007), therefore decreasing habitat complexity. Our findings agree with Lee (2006) in the fact that the increase of only one form-functional group (fleshy macroalgae) decrease the habitat complexity provided by coral, moreover we add that the increase of only a couple of species, within the fleshy macroalgae, will result in a loss of complexity provided by the diverse types of macroalgae present in a "healthy" reef system. The different scales at which each macroalgal group adds complexity will affect organisms functioning at such scale of complexity (Dahl, 1973). How the loss of complexity will impact the interaction with the following trophic level need to be addressed in long-term designed experiments (Toohey et al 2007), however we can assume that this loss can affect settlement (Lecchini et al 2007) and post-settlement (Eggleston 1995) of corals and survivorship of coral reef fishes (Beukers and Jones 1997).

The morphometric results showed that small size, erect highly branched and dense growth are more frequent in the reef compared to the lagoon where larger, erect, less branched and less dense algae are found. These morphological characters could be correlated to different environmental settings of water movement and substrate in the reef and lagoon at Puerto Morelos, since it is known that water movement differ through sections of Puerto Morelos coral reefs (Jordan 1979). In this regard, the small size, dense growths observed in reef species might be related to phenotypic plasticity to each site's hydrodynamic conditions, as turf-forming algae can tolerate and reduce water velocities (Hurd and Stevens 1997). This hypothesized hydrodynamic plasticity is especially evident for species found in both environments, for example Collado-Vides and Robledo (1999) described morphological and physiological plasticity in *Caulerpa cupressiodes* in Puerto Morelos reef system, where short and compact growth was found in the reef and larger less compact growth in the lagoon, a well recognized clonal strategy. Morphological characteristics, such as frond size, have been shown to affect dislodgment and survival in high-turbulence environments, such as intertidal zone (Carrington 1990).

Due to the karstic nature of the system, underground runoffs as well as isolated springs are the sources of nutrients from the watershed. We could expect a higher nutrient concentration on particular sites of the lagoon at localized springs, however our data showed that in general all sites in the lagoon had similar species composition, and the only strong difference was between reef *vs.* lagoon; therefore we are confident that the differences found, related with the goals of this study, are associated with the main significant difference between the reef and lagoon: substrate, water movement and light. An alternative explanation is that our sites were far away from springs and a direct effect of higher nutrient concentration was avoided. We do recognize that a more detailed study could detect differences in the macroalgal community as a function of nutrient sources as detected in other reef systems (Lapointe 1999). Particular algal morphologies have been related to nutrient uptake abilities (Littler and Littler 1980), and suggested to be used as potential indicators of nutrient sources in tropical systems (Fong et al 2001). Our results show a dominance of rhizophitic forms in the lagoon suggesting that macroalgae in the lagoon use nutrients from sediments, while *Laurencia* and other red attached species were found in the reef suggesting they have water column source (Fong et al 2001). Conducting a more detailed study including a survey of nutrient content of the different morphologies of macroalgae in order to detect sources of nutrients on the study site and its effects on the algal community is recommended.

This study show a high level of complexity in Puerto Morelos reef system, for the period studied, and in all categories used: number of species, number of functional-form groups and at the architectural level. In order to recover declining reefs and increase resilience in altered systems, we need to understand that macroalgae play different roles including being structural organisms.

Acknowledgement

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Mapping bottom features of the site selected for the Underwater Observatory in Sharm el Sheikh (South Sinai, Egypt)

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Abstract. As part of the "Pilot project for the sustainable development of environmental sound management in South Sinai, Sharm el Sheikh, Ras Mohammed National Park" funded by the Italian Environmental Ministry we undertook underwater visual, photographic and video surveys along 15 belt transects. A bionomic and topographic map (scale 1:100) of the site selected for construction of an Underwater Observatory was made as part of the building plan of the structure.

Key words: Mapping, Belt transect, Coral reef, Red Sea, Egypt.

Introduction

Cartography of the sea bottom plays a key role in coastal management. Knowledge of the distribution of the main marine biocenoses is of fundamental importance in conservation and monitoring programs (Piazzi et al. 2000). The creation of habitat or geomorphology maps is a critical step towards the assessment and management of reef ecosystems (Andréfouët and Guzman 2005). Current applications of coral reef habitat maps include biogeochemical budgets (Andréfouët and Payri 2001) or resource assessment and exploitation planning (Long et al. 1993; Andréfouët et al. 2004). An interesting recent application is to use remotely sensed habitat maps as indirect guides for assessing biological diversity in the context of marine conservation, or to identify the scale of processes that controls the structure of a mosaic of habitats (Mumby 2001).

The present study was part of the "Pilot project for the sustainable development of environmental sound management in South Sinai, Sharm el Sheikh, Ras Mohammed National Park" funded by the Italian Environmental Ministry. Within this project the realization of a bionomic and topographic map of the site selected for construction of an Underwater Observatory was proposed in order to minimize direct impact on benthic organisms.

Material and Methods

The study area was located at Marsa Ghoslani in front of the Visitor Center of the Ras Mohammed National Park Park, Sharm el Sheikh (Egypt).

Due to the limited dimension of the area to be investigated and the high level of detail needed, it was decided to operate using a direct method of survey instead of indirect methods like those based on acoustic instruments or satellite images, better suited for broader scale investigation.

Visual, photographic and video surveys were made by SCUBA diving along 6m wide x 50m long belt transects (n=15) perpendicular to the coastline (Bianchi et al. 2003; Hill and Wilkinson 2004) (Fig. 1).

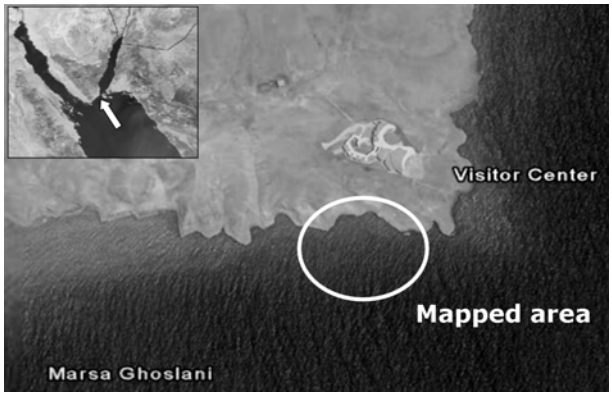

Figure 1: Study location along the coast of Sharm el Sheikh, Egypt.

The transects started from the reef crest and each starting point was positioned and georeferenced using a dGPS.

Visual recording of bottom features included type of substratum (rock, sand and rubble) and type of organisms (hard coral, soft coral). Bathymetric data were obtained measuring depth at each metre along the lines. Data were gridded using SURFER v. 8.0 (Golden Software, Inc.) to obtain the map.

Results

A bionomic and topographic map was obtained of a portion of marine bottom of about 5.000 m^2 (scale 1:100; reference point at 27°49.55'N, 34°16.14'E) (Fig. 2).

The map shows a fringing reef of only a few meters width. The reef flat is not continuous along the mapped coast, the interruption is visible on the map as the light grey area between the darker areas representing the fringing reef. When present, the reef descends from the crest to a depth of about 5 meters reaching a rocky platform covered by patches of sand, rubble and scattered living corals. In the mapped area this platform reaches depths of about 15-18 meters with a mean inclination of about 20%. The map clearly shows the presence and the distribution of living corals and some columns covered by a rich benthic community.

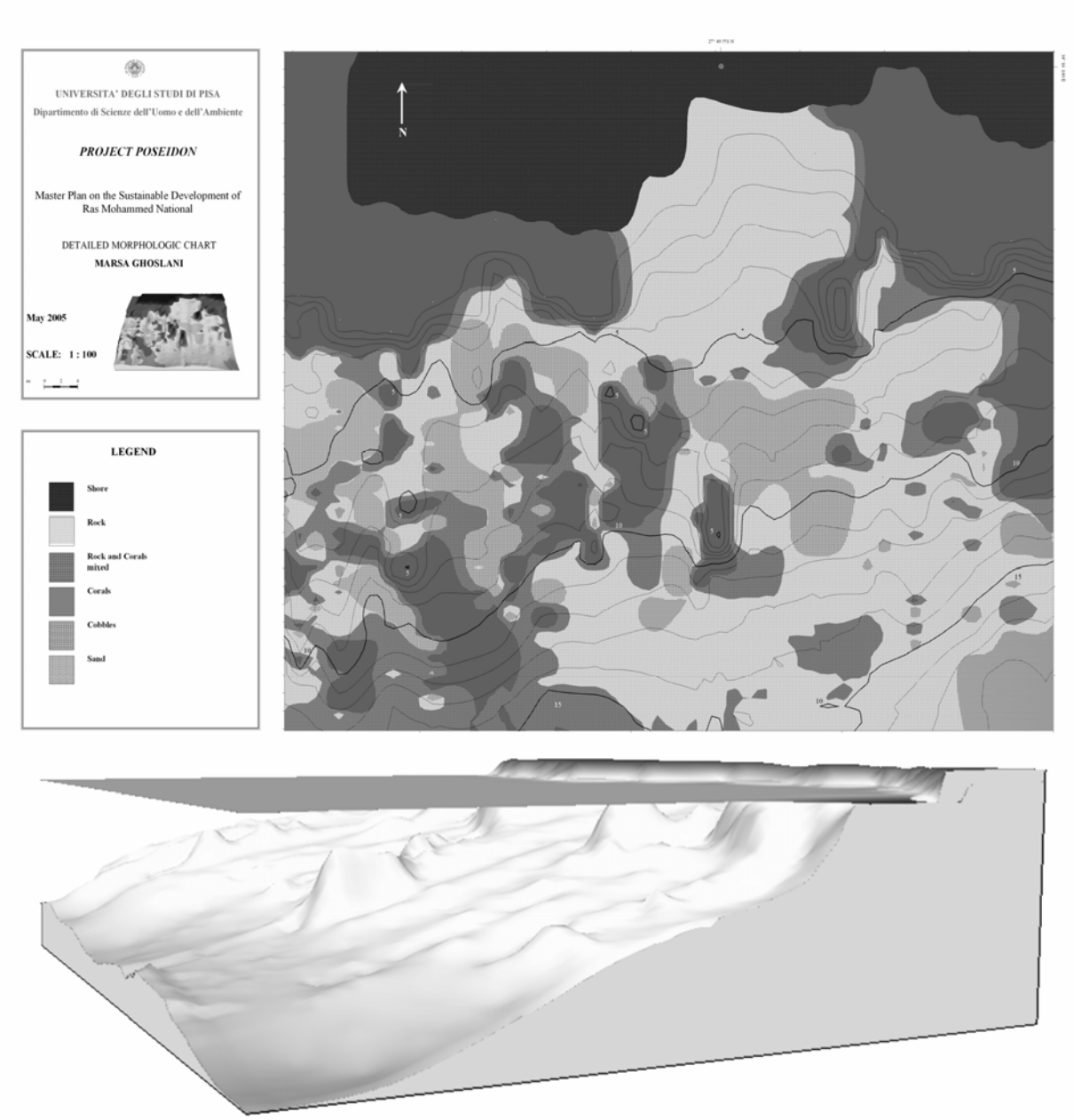

Figure 2: Bionomic and topographic map and three-dimensional representation of the bottom.

Discussion

Mapping bottom features is one of the essential steps to address environmentally sustainable solutions for underwater construction works. Of course, the knowledge of the extension and distribution of living corals must be considered as only part of the information necessary to manage this resource (Piazzi et al. 2000).

The map obtained from this study was used to elaborate the building plan of the Underwater Observatory. Suggestions were made regarding the type and shape of the structure and the route that it had to take in order to minimize direct impact on benthic organisms.

The Underwater Observatory consists of 3 structures: the underwater pipe-shaped tunnel, 58m long and with a diameter of 3m; the tower-lift, with a diameter of 6.5m that links the tunnel to the surface and that is connected to the coastline through a wharf 20m long and 5m wide.

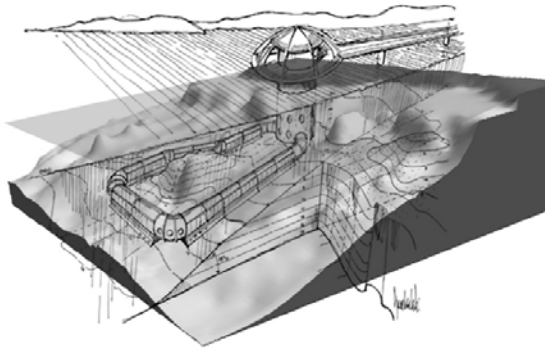

Figure 3: Sketch of the Underwater Observatory.

As can be seen in the sketch in Fig. 3, thanks to the map obtained, the ideal concept of the project plans to locate the underwater tunnel on the rocky bottom present in the area, thus avoiding the pinnacles covered by living corals. Moreover, it was possible to locate the wharf and the tower-lift where the fringing reef is naturally interrupted.

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Distribution, Abundance and Volume of *Xestospongia muta* at Selected Sites in the Florida Keys National Marine Sanctuary

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Abstract. In 1994, the Florida Keys Coral Reef Evaluation and Monitoring Project (CREMP) was initiated to provide status and trends data for the benthic reef resources of the Florida Keys National Marine Sanctuary (FKNMS). Forty sampling sites were selected within the FKNMS. Permanent station markers were installed in 1995 and annual sampling began in 1996. In 2007, an ongoing clionid sponge survey was expanded to include the barrel sponge, *Xestospongia muta,* at all CREMP monitoring stations.

The *Xestospongia muta* sampling method was developed based on existing CREMP project station layout with three 1-meter-wide belt transects providing the maximum spatial coverage within each station.

The distribution, abundance and volume of *X. muta* were analyzed sanctuary-wide, regionally and by habitat type for 103 sampling stations. Sanctuary-wide, 89% of abundance and 93% of volume were observed at offshore deep sites. At offshore shallow and patch sites 6% and 5% of total abundance and 5% and 2% of total volume were recorded respectively. *Xestospongia muta* was not observed at any hardbottom site sanctuary-wide. At the regional level, while sponges the Middle Keys had the greatest abundance and volume, the largest individual sponges were recorded in the Upper Keys.

Key Words: Florida, reef tract, sponge, *Xestospongia muta*

Introduction

Sponges are the most abundant sessile organisms in terms of biomass in many Caribbean reef communities (Diaz and Rützler 2001), and sponge species diversity may exceed that of corals (Wulff 2001). Sponges play many important roles in the reef ecosystem: filtering bacteria and nutrients from the water column, binding corals to the substrate, facilitating reef regeneration, and providing a food source for spongivores (Diaz and Rützler 2001; Wulff 2001). Sponges are also major competitors of corals in terms of reef space. Historically, data collection on sponges has been neglected in monitoring projects because sponge sampling poses a myriad of challenges to researchers. Field identifications can be difficult and sponge systematics is in flux on all taxonomic levels (Wulff 2001).

Since its inception in 1996, the Coral Reef Evaluation and Monitoring Project has documented stony-coral species richness through underwater inventories and percent benthic cover through video transects. These surveys are carried out at permanent stations throughout the Florida Keys National Marine Sanctuary. In 2001, the CREMP began monitoring the abundance and area of bio-eroding sponge species at all CREMP stations. The three clionid sponge species (*Cliona delitrix, C. lampa*, and *C. caribbaea*) recorded by the CREMP are known to be aggressive coral bio-eroders and over-growers (Callahan et al. 2007). In 2005, a fourth clionid, *Cliona varians* (formerly *Anthosigmella varians*), was added to the survey. In 2007, the barrel sponge, *Xestospongia muta* was added.

Xestospongia muta is commonly found on coral reefs and in subtropical hardbottom communities typically at depths greater than 10 m. This sponge is conspicuous and can be accurately identified in the field. It contributes greatly to overall sponge biomass due to its tremendously large size. Oscula diameters have been recorded as wide as 2.5 meters (Nagelkerken 2000). Populations of this species occupy greater than 9% of the available reef substrate in some regions (Zea 1993). Barrel sponges also have a tremendous filtering capacity and are an important source of inorganic nutrients. Sponges, including *X. muta*, host many prokaryotic (especially cyanobacteria) and eukaryotic symbionts and contribute significantly to reef productivity (Wilkinson 1983). *Xestospongia muta* is studied extensively for its secondary metabolite production. These sponges exhibit bleaching which, as in coral, may become fatal. Therefore

monitoring these sponges can be used as a metric for monitoring reef-community health. Mean specific growth rates as fast as 4.04 $year^{-1}$ and as slow as 0.02 $year^{-1}$ have been observed (McMurray et al). Specific growth rates of *X. muta* have been found to decrease with increasing sponge volume according to McMurray et al.

Including *X. muta* in the expanded CREMP sponge survey will provide data on the distribution, the number of individuals, and the size of these individuals in terms of volume. Changes in these values can be monitored over time at all of the CREMP stations throughout the Florida Keys National Marine Sanctuary. These data will help to elucidate the population dynamics, community structure, and health of these important and often understudied reef organisms.

Material and Methods

The same survey method was employed at each CREMP site. Thirty-eight sites (Fig. 1) comprised of 103 sampling stations were surveyed for *X. muta* in this 2007 study. The sites represent four habitat types: offshore shallow reefs, offshore deep reefs, patch reefs, and hardbottom communities. The CREMP sampling within the FKNMS encompasses three regions defined as Upper Keys (north Key Largo to Conch Reef), Middle Keys (Alligator Reef to Moser Channel), and Lower Keys (Looe Key to Smith Shoal) (Beaver et al 2006).

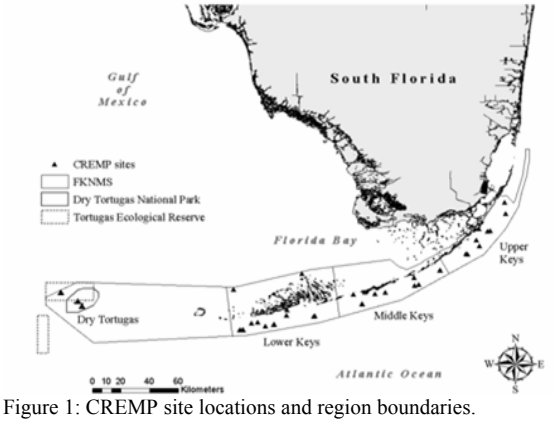

Figure 1: CREMP site locations and region boundaries.

Each sampling station within a site is permanently marked with two stainless steel reference stakes. Two steel poles, three plastic chains, and three measuring tapes mark a station's boundaries and define sampling transects (Fig. 2).

The sponge survey area is defined by three 1 meter wide belt transects within an existing CREMP station (Fig. 2). The survey area is delineated by a diver who swims directly above the transect tape and holds a meter stick perpendicular to the tape and parallel to the reef surface. The total station survey area is approximately $66m^2$. The diver records the location and volume of each *X. muta* encountered as well as notes on bleaching, disease, and scleractinian interactions. Every sponge observed can be relocated within the sampling station.

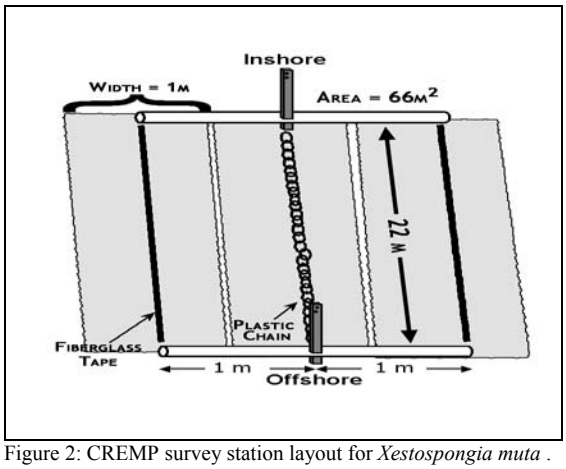

Figure 2: CREMP survey station layout for *Xestospongia muta* .

Because of the morphological plasticity of this sponge, volume was measured by approximation to various geometric solids such as cubes, prisms, cones, and spheres (Wulff 2001). The frustum, or truncated cone usually approximated the shape of *X. muta* most accurately. The radius of the apex (R1) and radius of the base (R2) were measured along with the height of the sponge (H1). These measurements were calculated as the volume of the frustum. The radius of the oscula (R3) and the depth of the oscula (H2) were also measured. The volume of this cone was subtracted from the volume of the frustum to provide a more accurate total sponge volume. The final equation for frustum volume calculations was:

$$1/3\Pi*H1[(R1^2 + R2^2) + (R1*R2)] - 1/3\Pi R3^2*H2$$

A custom designed underwater datasheet explicitly designed for this survey allowed for measurements to be recorded easily and accurately. Larger sponges were approximated to inverse frustums or cylinders. In 2008, underwater maps were created to relocate sponges previously sampled and identify new recruits at CREMP stations.

Results

Data on the distribution, abundance and volume were analyzed sanctuary-wide, regionally and by habitat type for 103 sampling stations. One CREMP station equals one sample.

Sanctuary-wide Distribution by Habitat Type

Sanctuary-wide, 89% of abundance was observed at offshore deep sites, while sponges at offshore shallow and patch sites made up 6% and 5% of abundance respectively (Fig. 3). Offshore deep sites contained 93% of total sponge volume, while sponges at offshore shallow and patch reefs contributed only 5% and 2% respectively (Fig. 4). No *X. muta* were observed at any of the three hardbottom sites sanctuary-wide.

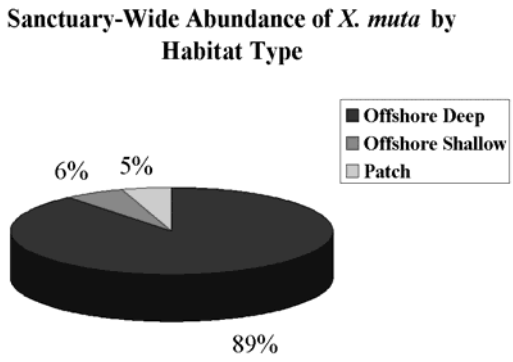

Figure 3: Sanctuary-wide abundance of *X. muta* by habitat type. No individuals were found at any CREMP hard-bottom stations.

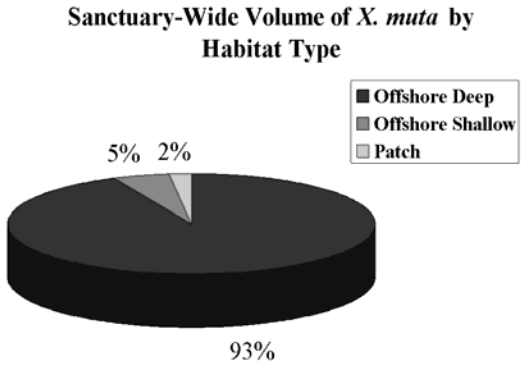

Figure 4: Sanctuary-wide volume of *X. muta* by habitat type.

Abundance: Regionally and by Habitat Type

At the regional level, sponges at sites in the Middle Keys (29 stations) contributed the greatest mean (+/- 1 SE) abundance (0.051 +/- 0.015 individuals/m^2). Abundance ranged from 0.015 individuals/m^2 at several stations to 0.333 individuals/m^2 at Tennessee Deep station 4. This station had the greatest *X. muta* abundance of all those surveyed by the CREMP. Sponge abundance recorded in the Upper Keys (28 stations) was 0.023 +/- 0.011 individuals/m^2, while the abundance in the Lower Keys (46 stations) was 0.046 +/- 0.012 individuals/m^2 (Fig. 5)

When abundance is examined by habitat type, the greatest abundance (0.172 +/- 0.043 individuals/m^2) was at offshore deep sites in the Middle Keys. The Upper Keys deep sites had the least sponge abundance (0.101 +/- 0.036 individuals/m^2), while the Lower Keys deep sites had a sponge abundance of 0.143 +/- 0.021 individuals/m^2 (Fig. 6).

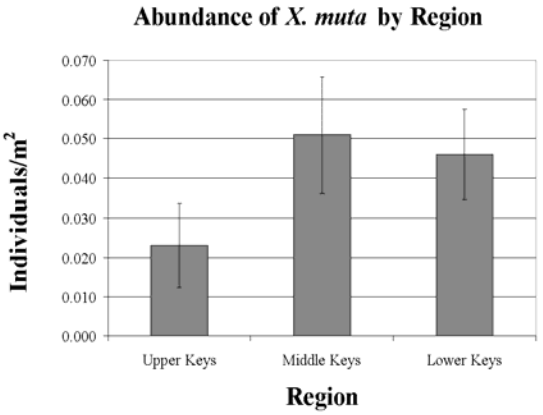

Figure 5: Abundance of *X. muta* by region

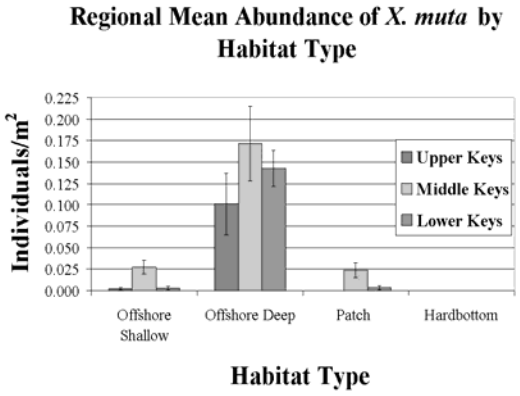

Figure 6: Regional mean abundance of *X. muta* by habitat type. No individuals were recorded at Upper Keys' patch reefs

Volume: Regionally and by Habitat Type

In all regions offshore deep sites had the highest mean (+/- 1 SE) sponge volume. The Middle Keys' deep stations had the greatest sponge volume (2100 +/- 1057 cm^3/m^2). The Upper Keys' deep reefs had the next highest sponge volume (1609 +/- 624 cm^3/m^2). The Lower Keys' deep reefs had the least *X. muta* volume with 960 +/- 172 cm^3/m^2 (Fig.7). The lowest sponge volume by station was 8.31 cm^3/m^2 at Sand Key Deep station 3, and the greatest sponge volume by station was found at Tennessee Deep Station 4 with 7207 cm^3/m^2. The largest individual sponge was recorded at Molasses Deep station 1 (133,070 cm^3), while the smallest

individual sponge was recorded at Eastern Sambo Deep station 3 (1.05 cm^3). The largest individuals by sponge volume were at Upper Keys' deep stations (Fig. 8).

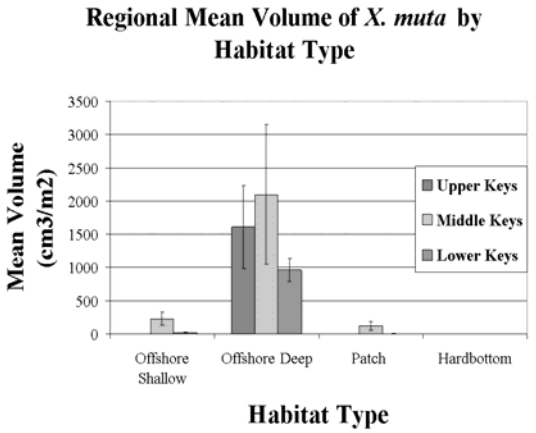

Figure 7: Regional mean volume of *X. muta* by habitat type

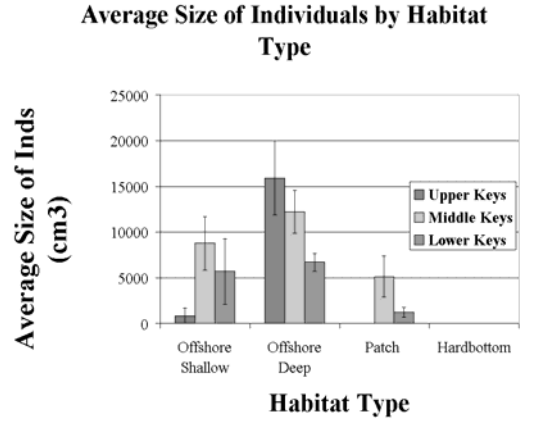

Figure 8: Average size of individual *X. muta* by habitat type

Surface area

When sponge data for all CREMP stations are averaged, the surface coverage of *X. muta* is under 1%, but when only data for the offshore deep sites are considered, surface area increases. The average *X. muta* coverage at offshore deep sites is 1.26%. The range varies from 1.71 cm^2/m^2 or 0.26% to 295 cm2/m2 or ~4.5% at Tennessee Deep station 4.

DISCUSSION

Xestospongia muta is Most Abundant at Deep Sites

In the present effort we have surveyed offshore shallow reefs, offshore deep reefs, patch reefs, and hardbottom habitats for the presence of the barrel sponge, *Xestospongia muta*. We obtained measurements on the number of individuals and volume to describe the population. This initial survey will serve as a baseline year for additional years of monitoring and further research projects.

It was expected that sponge abundance and volume dominated the FKNMS at the offshore-deep reef sites. Sponges at Middle Keys' deep sites averaged 0.172 individuals/m^2. *Xestospongia muta* is typically found at depths greater than 10 meters. The CREMP offshore-deep sites are the only sites sampled with these depths. However, *X. muta* was not present at every offshore deep site sampled. In the Upper Keys none were observed at the two stations comprising the Carysfort deep site. This was the only deep site sampled that had no *X. muta*. The depth range at this site is between 12.5 m and 16.2 m which is a typical depth range of *X. muta* habitat. Additional research on water-quality conditions and ocean currents may provide insight as to the absence of the sponge at this site. At Western Sambo deep in the Lower Keys, *X. muta* was present at two stations with a depth of 12 m, but absent at the third station, which had a shallower depth of 6.7 m. Nutrients and particles in the water column at this shallow station may be insufficient to support these large sponges. There are observational data for the presence of *X. muta* at shallow reefs (less than 5 meters in depth) in the Caribbean (Smithsonian Tropical Research Institute 2003). Data recorded in this study from offshore shallow reef sites (Fig. 6) support those observations. Shallow reef sponges were less barrel-shaped than those at deep sites, but were vase-shaped and usually projecting out from under an overhang. Few sponges were recorded at patch reef sites (0.007 individuals/m^2), but at several non-CREMP patch sites (less than 10 meters in depth) several barrel sponges were observed (M. Bertin pers. obs.).

Sponge volumes in this study ranged from 1.05 to 133,070 cm^3. This range is similar to those (24.05 to 80,281.67 cm^3) observed by McMurray et al. *Xestospongia muta* covered an average of 1% of available reef substrate at CREMP stations, but this sponge covered 4.5% of available substrate at Tennessee Deep station 4. This cover value is about half of that reported by Zea in 1993 (>9%).

No observations of bleaching and disease were recorded in this 2007 study. However, spots determined to be grazing scars were observed on several individuals. Parrotfish are known grazers of *X. muta* and the grazing is increased on those sponges that have bleached (Dunlap and Pawlik 1998). This may be from reduced chemical defenses in the bleached sponge (Dunlap and Pawlik 1998). Additional years of study may provide greater knowledge of in situ bleaching, disease, and grazing identification. Several sponges were observed with large holes which were surmised to be damage from anchors.

This study showed that the greatest abundance and volume of *X. muta* is at the CREMP offshore deep reef sites (Figs. 3 and 4, 14 expected individuals/100 m^2 at deep CREMP sites versus 0.9 and 0.7 individuals/100m^2 at offshore shallow and patch sites).

Acknowledgements

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Impacts of coastal development on ecosystem structure and function of Yucatan coral reefs, Mexico

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Abstract. We report the results of a multi-site comparative analysis of coral reef condition along a gradient of coastal development in the Yucatan Peninsula (Mexico). Benthic surveys and fish counts performed in 2000 were used to assess ecosystem structure and functional reef components in three regions of the State of Quintana Roo: 1) the Riviera Maya in the north, with heavy tourist development; 2) the Sian Ka'an Biosphere Reserve, a protected area with very low human density; 3) the Costa Maya in the south, with relatively little tourist development. Results showed significant differences in the ecological state of coral reefs along the coast. Northern reefs exhibited an algal-dominated state with a very low coral cover, whereas southern reefs exhibited a more balanced coverage of macroalgae and corals with extended areas of free substrate. No clear trends were apparent in the spatial patterns of the biomass of herbivorous fishes, but fish functional diversity decreased from south to north. Results suggest that massive coastal tourism development combined to a low resilience may have caused major shifts in the structure and function of coral reefs in the northern region of the Yucatan Peninsula.

Key words: Macroalgae, Herbivorous fish, Functional diversity, Tourism, Disturbance.

Introduction

Caribbean coral reefs have been suffering a regional-scale decline as a result of multiple disturbances (Gardner et al. 2003, Wilkinson 2004). Increasing coastal development calls for a better understanding of the key processes driving coral reef degradation since the causes and mechanisms leading to this decline are still debated (Aronson and Precht 2006, Mora 2008). In addition, reef management requires functional approaches to understand how disturbances affect the structure and function of coral reefs (Hughes et al. 2003, Bellwood et al. 2004).

The Mesoamerican barrier reef system is home to some of the richest biodiversity in the wider Caribbean, but it is threatened by intensive coastal development induced by fast-growing tourism industries (Kramer and Kramer 2002, Wilkinson 2002, 2004). Along the Caribbean coast of the Yucatan Peninsula (Mexico), the State of Quintana Roo has endured a massive tourism development, starting on Cancun in the 1970s and extending towards the south during the last three decades. Given the strong anthropogenic pressure, the ecological state of Quintana Roo coral reefs needs to be evaluated to search for possible impacts of coastal development.

We report the results of a multi-site comparative analysis of coral reef condition along the Quintana Roo coast. A functional approach, based on the assessment of key functional components of benthic and fish communities, was developed to evaluate the potential impacts of anthropogenic disturbances.

Material and Methods

Study area

Mexican Caribbean coral reefs extend for over 400 km along the coast of the State of Quintana Roo on the eastern side of the Yucatan Peninsula (Fig. 1).

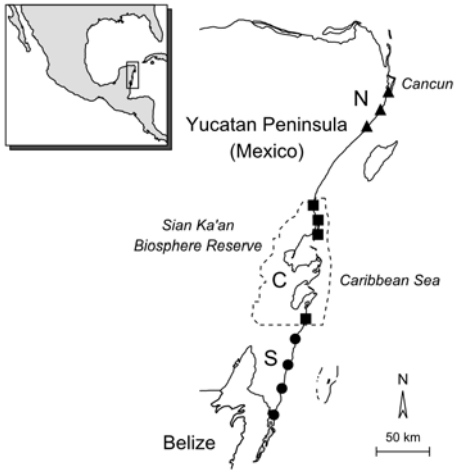

Figure 1: The 11 study sites in the northern (N, triangles), central (C, squares) and southern (S, circles) Quintana Roo coast.

Quintana Roo reefs are referred as extended fringing reefs and vary slightly from a gentle slope with low relief in the north to better developed reefs with spur and groove formations in the south (Jordán-Dahlgren and Rodríguez-Martínez 2003, Núñez-Lara et al. 2005). According to human use and coastal development, the Quintana Roo coast can be broadly divided into 3 regions: the Riviera Maya in the north (N) with heavy coastal development, especially in Cancun; (2) the Sian Ka'an Biosphere Reserve in the central region (C) with very low human density and a protected status; the Costa Maya in the south (S) with low human density and small but rising tourism development.

Data collection

Fish counts and benthic surveys were performed (Núñez-Lara 2003) in 2000 on the 6-m deep fore-reef zone of 11 reef sites distributed in the three regions N, C and S (Fig. 1). Fish censuses were conducted on 18 replicate 50 x 2-m belt transects and species biomass was subsequently estimated from individual size using length-weight allometric relationships compiled in FishBase (Froese and Pauly 2008). Benthic coverage was assessed from digital video imagery (Osborne and Oxley 1997), video transects being later analyzed using a point sampling method. A plastic sheet marked with 13 points was laid over a monitor screen, and sessile organisms and substrate types lying beneath each point were counted on 40 regularly spaced frames. For each video transect, percentage cover of benthic components was then estimated from 520 point counts.

Finally, we gathered socio-economic indices for the coastal municipalities (the second-level administrative division in Mexico) of the Quintana Roo state for the period 2000-2002. Urban areas and road density (SEPLADER 2006), human density (INEGI 2000) and number of hotels (SEDETUR 2008) were used to quantify the anthropogenic pressure along the Yucatan coast.

Data analysis

Percentage cover of fleshy macroalgae (canopy height >1 cm), live corals (scleractinians and gorgonians) and bare substratum (i.e., free hard substrate) was compared in the regions N, C and S of the Quintana Roo coast. Total fish biomass and fish herbivore biomass (*Scaridae* and *Acanthuridae*) were also compared, as well as the number of fish species per transect. Comparisons between the three regions were performed using pairwise t-tests with the Bonferroni correction. Finally, functional diversity (species richness by functional group) was investigated by pooling fish species into 28 functional groups defined by feeding habits, size and mobility. For each reef site, we estimated the number of fish species (SR) by functional group, and mean SR per group were calculated 1) over all reef sites (global mean SR) and 2) over the reef sites of each region (regional mean SR). Deviations of regional means to the global mean were analyzed for each functional group.

Results

Indices of coastal development

Socio-economic data indicated a clear gradient of coastal development along the Quintana Roo coast (Fig. 2). Urban areas and human density decreased strongly from north to south. In addition, tourism infrastructure was far more developed in the north: the number of hotel rooms reached 25,000 in Cancun and decreased to about 15,000 along the whole Riviera Maya, then 100 and 200 in the Sian Ka'an Biosphere Reserve and in the Costa Maya respectively.

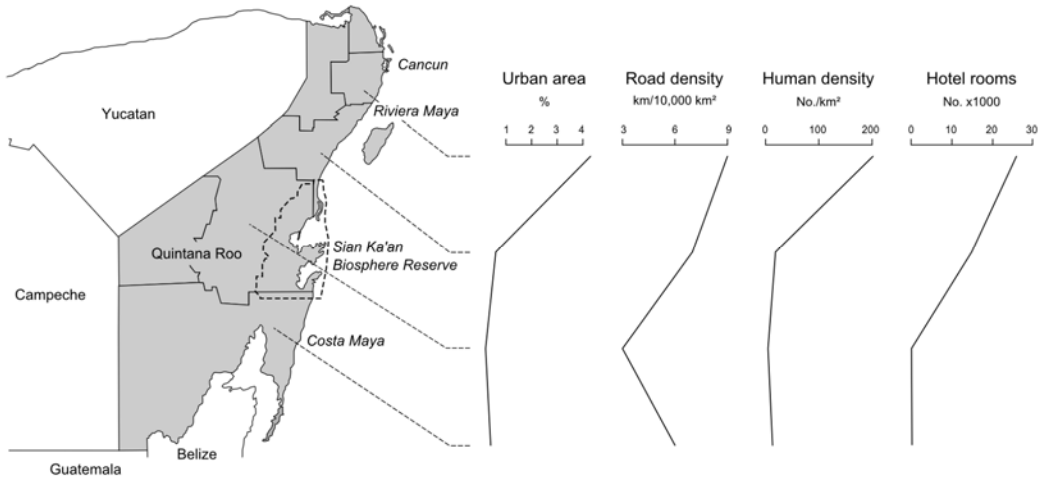

Figure 2: Indices of coastal development for the 4 coastal municipalities of the Quintana Roo state.

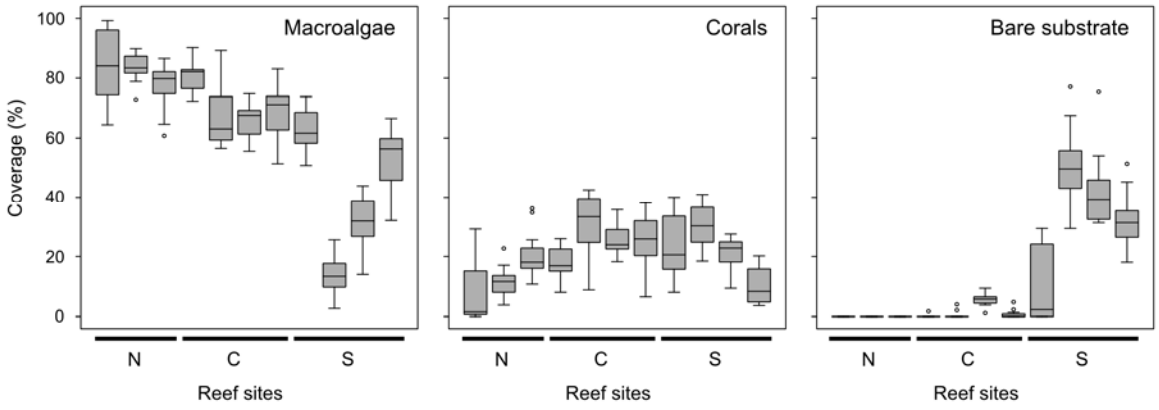

Figure 3: Benthic coverage of macroalgae, corals and bare substratum at the 11 surveyed reef sites of the Quintana Roo coast.

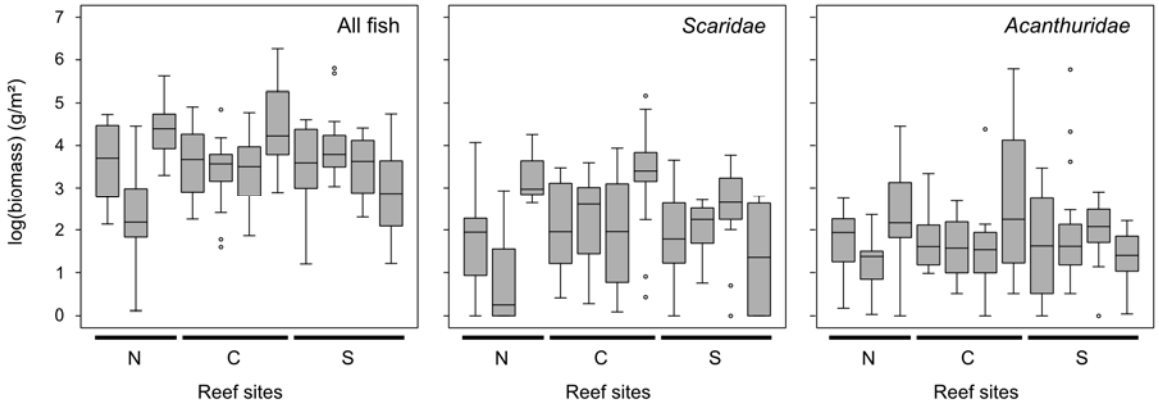

Figure 4: Total fish biomass and biomass of *Scaridae* and *Acanthuridae* at the 11 surveyed reef sites of the Quintana Roo coast.

Benthic cover

The percentage cover of macroalgae was higher in the north (Fig. 3, mean ± s.e. 82 ± 1%) and decreased significantly ($p<0.001$) in the central region (71 ± 1%), then in the south ($p<0.001$, 38 ± 3%). Maximum values were observed in the far north (10-km south to Cancun) where macroalgal cover reached 95% in several places. Coral cover was significantly lower ($p<0.001$) in the north (13 ± 1%) than the other regions (C: 25 ± 1%, S: 22 ± 1%). The coverage of bare substrate was null in the northern region and increased to the south where it reached one third (35 ± 3%) of the reef bottom.

Fish biomass

Total fish biomass (mean ± s.e. 58 ± 6 g/m²) was highly variable between and within reefs (Fig. 4) but did not vary significantly between the three regions. In average, herbivores accounted for 49% (± 3) of the total biomass of fish assemblages. Biomass of *Scaridae* was significantly higher in the central region compared to the north ($p<0.05$) and the south ($p<0.01$). The biomass of *Acanthuridae* did not vary significantly. As a result, herbivore biomass and macroalgal cover did not show a significant relationship along the Quintana Roo coast.

Fish diversity

Fish species richness (Fig. 5) was significantly lower ($p<0.01$) in the north (11.4 species/transect) than the central region (14.3 sp.) and the south (15.5 sp.).

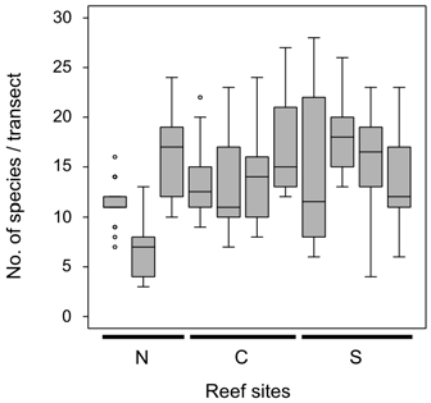

Figure 5: Fish species richness along the Quintana Roo coast.

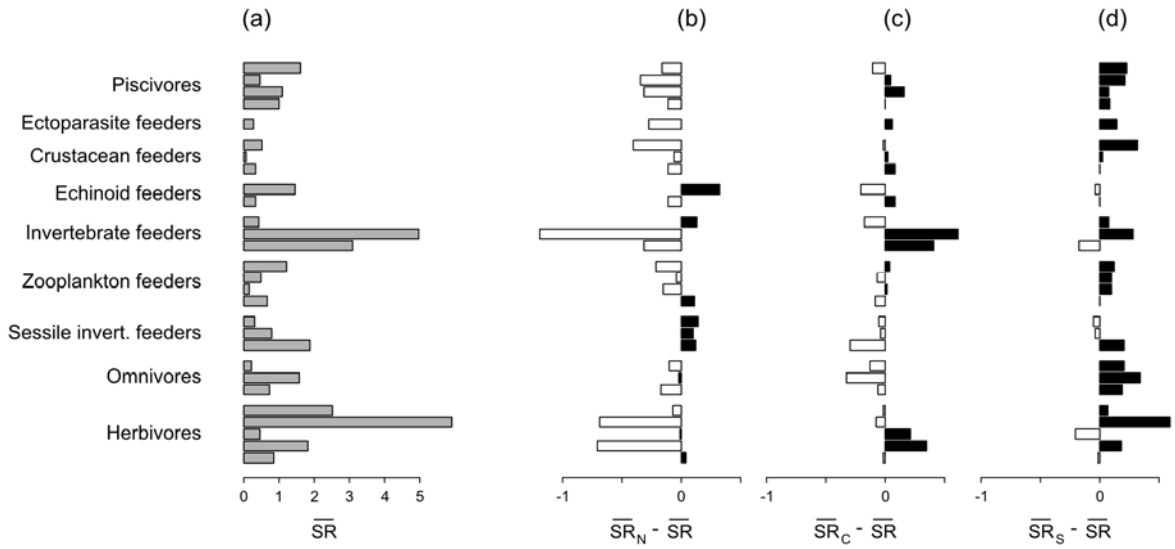

Figure 6: Fish functional diversity along the Quintana Roo coast described by (a) the global mean species richness (SR) of the 28 functional groups and deviations from the global mean SR to (b) northern, (c) central and (d) southern mean SR.

Fish functional diversity also increased from north to south (Fig. 6). Species richness of some herbivore, invertebrate feeder and high-level carnivore groups increased. Inversely, some functional groups, such as echinoid feeders and sessile-invertebrate feeders, were more speciose in the north.

Discussion

In 2000, the benthic structure of coral reefs exhibited highly contrasted patterns along the Quintana Roo coast. Northern reefs were largely dominated by macroalgae (82% mean coverage) and live coral cover was very poor (13%), whereas southern reefs exhibited a more balanced coverage of macroalgae and corals (38% and 22% respectively) with extended areas of bare substrate available for colonization. Those patterns indicate considerable changes in the equilibrium between macroalgae and corals following a north-to-south latitudinal gradient. From southern to central reefs, macroalgae progressively overwhelmed the available free space, and then overgrew corals in the northern region until they dominated and saturated the reef bottoms. Those patterns indicate a latitudinal gradient of coral-to-algae phase shift along the Quintana Roo coast. This gradient of ecological states suggests a major shift along the coast in the processes driving the equilibrium between macroalgae and corals.

In the recent decades, many Caribbean reefs have suffered major declines in coral cover leading to a phase shift from hard coral to fleshy algae dominance (Gardner et al. 2003, Pandolfi et al. 2005). Coral-to-algal phase shifts have been attributed to the reduction of herbivory as a result of diseases (mass mortality of the echinoid *Diadema antillarum*) and overfishing (Hughes 1994, Jackson et al. 2001, Mumby et al. 2007). From north to south, no clear trends were apparent in the biomass of herbivorous fishes on Yucatan reefs. Spatial information of the fishing pressure is scarce, but *Scaridae* and *Acanthuridae* are seldom targeted by Mexican fishermen. In addition, *Diadema* urchins did not recover in 2000 from the 1983-84 mass mortality event as only few isolated individuals were detected on videotransects. As a result, fishing pressure and herbivory are unlikely to have caused the benthic shifts observed along the Quintana Roo coast.

Other explanations of coral-to-algal phase shifts involve coastal development and associated nutrient enrichment (Lapointe 1997). In 2000, the Quintana Roo coast exhibited clear patterns in coastal development that fitted very well with the gradient depicted on the benthic structure. From the poorly developed coast in the south to the heavy touristic spot of Cancun in the north, urbanization, coastal population and tourism infrastructure sharply increased parallel to the latitudinal gradient of macroalgal proliferation. This suggests that tourism-driven coastal development may be the primary cause of coral-to-algal phase shifts detected along the Quintana Roo coast.

Other processes such as hurricanes and coral mortality have the potential to inverse the equilibrium between corals and algae (Ostrander et al. 2000, Aronson and Precht 2006). Coastal development may have affected reef resilience on Quintana Roo reefs and increased their susceptibility to natural disturbances. Ecosystem responses to disturbances vary according to ecosystem functions performed by organisms, and functional diversity and redundancy

(i.e., the number of taxonomically distinct species that exhibit similar ecosystem functions) can be expected to strongly influence coral reef susceptibility to coral/algal phase shifts (Bellwood et al. 2004, 2006). Our results showed a lower functional redundancy in northern fish community of some key functional groups, such as herbivores and piscivores. This may indicate that northern reefs were more susceptible to anthropogenic disturbances, or that benthic shifts have impacted the species composition of fish assemblages (see also Arias-González et al., in press). Our data do not allow to conclude if the spatial patterns of functional diversity are the result or the partial cause of the ecological changes depicted on the reef benthic structure. They suggest however that the potential of reef resilience is probably lower in northern reef in face to disturbances.

In 2000, macroalgae dominated northern reefs of the Yucatan Peninsula, and coral/algal phase shifts extended towards the south. During the last decade, coastal development has grown faster, stretching from Cancun to the northern edge of the Sian Ka'an Biosphere Reserve. The expanding costal development and the low redundancy of some functional groups are unlikely to have helped northern reefs to recover. In addition, tourism economy has reached southern reefs with the construction of a cruise ship pier for the development of tourism industry in the Costa Maya. Management actions are needed to protect the last healthy reefs of Quintana Roo coast which face a massive tourism development. As reported in the present study, coral cover was higher in some reef sites of the Sian Ka'an Biosphere Reserve, despite an algal-dominated state. Such patterns may encourage the development of protected areas to help coral reefs to maintain a substantial coral cover. Finally, this study provides a reference point of coral reef condition along the Quintana Roo coast, and future management of coral reef resilience may be improved by continued assessment of ecosystem metrics and functional attributes of species.

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Was the 1998 Coral Bleaching in the Southern Seychelles a Catastrophic Disturbance? - 1999-2006 Reef Fish Responses to Coral Substrate Changes

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Abstract. The southern Seychelles Islands suffered extensive bleaching-related losses of live coral habitat in 1998. The Aldabra Marine Programme investigated coral-bleaching-related responses in reef fish communities at the UNESCO-protected Aldabra Atoll and neighboring unprotected Assomption, Astove and St Pierre. These were all coral-rich systems before up to 50% loss of live corals. Hard coral recovery has been minimal except at St. Pierre. Habitat phase shifts have occurred only at Aldabra (soft coral, *Rhytisma*) and at Assomption (macroalgae, *Halimeda*). Fish abundances by species at Aldabra were different between sites and years, but there was no directional change within sites. Multivariate comparisons of fish assemblages at 10m and 20m depths showed no directional changes over time at Aldabra (1999-2006), Assomption (2002-2006), Astove (2002-2005), and St Pierre (2002-2005). At Aldabra, pre-bleaching reef fish community structure persists, and abundance changes appear to represent the "natural" system. Similar patterns at neighboring unprotected islands suggest any anthropogenic impacts are below the thresholds affecting local reef-resident species. Losses of live coral in these coral-rich communities apparently were not catastrophic disturbances that pushed these ecosystems beyond their "tipping point" which would reduce resilience and lead to changes in the basic structure of the reef fish assemblages.

Key Words: Words: bleaching, fish assemblages, catastrophic disturbance, tipping point

Introduction

The 1998 El Niño-related warm water event resulted in mass losses of corals due to bleaching that altered reef habitats on a historically unprecedented global scale (Hoegh-Guldberg 1999, Wilkinson 2004). The western Indian Ocean suffered some of the most severe bleaching-related coral mortality during this 1998 event, with up to 90% of the live corals in the region affected by bleaching (Wilkinson et al. 1999). In the southern Seychelles Islands there were extensive bleaching-related losses of live coral habitat on the outer reefs, with coral mortality ranging from 60-75% and losses of live coral cover as high as 60% (Teleki et al. 1999, Spencer et al. 2000, Spalding and Jarvis 2002, McClanahan et al. 2007). Aldabra Atoll and the neighboring southern Seychelles islands of Assomption, Astove and St Pierre were coral-rich systems prior to losses of up to 50% of live coral in the 1998 bleaching-event (Spalding and Jarvis 2002, Stobart et al. 2005, Downing et al. 2006). A 1998 survey of the outer reef scleratinians at Aldabra Atoll during the late stages of the bleaching event found 36-100% recently bleached or dead depending on depth (Spencer et al. 2000).

Losses of live coral of this magnitude often result in decreased structural heterogeneity of the reef habitat through erosion of the dead coral framework. The associations between reef fishes and coral habitat complexity (review in Wilson et al. 2006) support the conclusion that reef fishes should be affected by mass losses of corals. However, studies of the responses of fishes on different reefs from 1-6 years after the 1998 mass bleaching have yielded mixed and differing results (Spalding and Jarvis 2002, Bellwood et al. 2006, Garpe et al. 2006, McClanahan 2006).

In a controlled experiment Holbrook et al. (2006) found that the amount of live coral habitat can influence fish assemblages, including fishes that have generalized habitat requirements (i.e., non-coral-obligate species). Further, this experiment suggested that on coral-rich reef habitats there must be an almost catastrophic loss of live coral cover to substantially change the basic structure of the fish assemblage.

Here we use results of long term monitoring of the coral reef communities at Aldabra Atoll and the neighboring islands of Assomption, Astove and St. Pierre to assess responses of the associated fishes to the extensive and massive 1998 bleaching-event losses of live coral. These four locations were coral-rich systems prior to 1998. We investigate if changes in the coral habitat in 1998 represented catastrophic disturbances for local fish assemblages. The surveys at one protected and three unprotected locations provide information on responses of local fish populations to any synergy between bleaching and direct anthropogenic or fishing impacts.

Methods

Quantified digital videography surveys of the benthic habitats, and quantified visual surveys of species and size-groups of fishes, were conducted by the Aldabra Marine Programme (AMP) on the outer reefs at Aldabra Atoll, and Assomption, Astove and St. Pierre Islands (Fig. 1). Surveys were along permanently marked transects on the 10 m and 20 m depth contours (for protocols see Teleki et al. 1999). Surveys at Aldabra Atoll conducted at Sites 1-4, 6 and 8 (Stobart et al. 2001) along the northern shore were used for this study. Site 5 had atypical coral rubble habitat, and Site 7 transect depths were 5m and 15m. Surveys at Aldabra were during November 1999, February 2001 and 2002, May 2003, March 2004 (incomplete surveys), April 2005 and December 2006. Surveys were conducted at one site each at Assomption, Astove and St. Pierre during February 2002, May 2003, March 2004 (incomplete surveys), and April 2005, and at Assomption during December 2006 (Stobart et al. 2002).

The habitat video imagery was analyzed using the AIMS 5-dot method (Osborne and Oxley 1997) and used to compare the annual percentage change in corals, algae and other habitat categories. PRIMER multivariate comparisons (MDS) were made of the abundance of fishes between years, tested for linear sequence (RELATE), and at Aldabra Atoll tested for differences between sites (ANOSIM; Clarke and Warwick 2001).

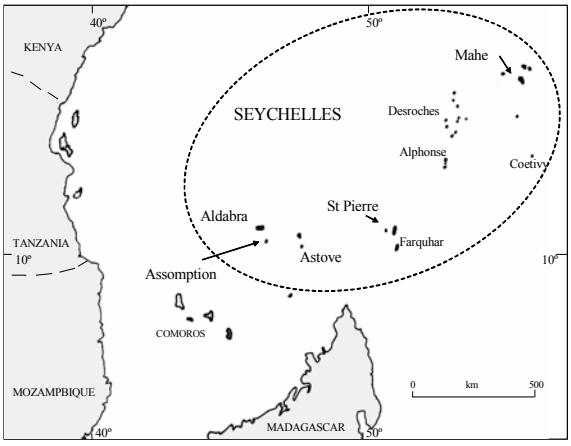

Figure 1: Location of AMP sites in the Seychelles, western Indian Ocean.

Results

Benthic habitats

At Aldabra Atoll the increases in live coral cover since the post-bleaching survey in 1999 were driven by extensive growth of the soft coral *Rhytisma* at both 10m and 20m (Fig. 2). There was little change in the growth of other soft corals at either depth. Recovery of hard corals was minimal at 10m and 20m. Macroalgal cover declined at both 10m and 20m to the lowest levels recorded in the eight years following the bleaching event. Erect dead coral habitat continued to decrease at both 10m and 20m (see AMP reports available at http://aldabra.org).

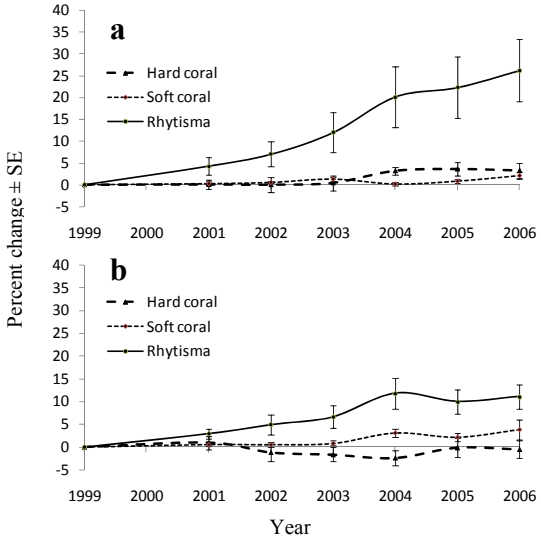

Figure 2. Change in hard coral, soft coral and *Rhytisma* at Aldabra atoll (see legend) at a) 10m and b) 20m depth between 1999 and 2006.

At Assomption, Astove and St. Pierre, the growth of *Rhytisma* and other soft corals was minimal, although relatively more pronounced at Assomption. The soft corals were not important factors in any increases in live coral cover at either 10m or 20m. Increases in hard coral cover were minimal at Assomption and Astove, but relatively large and steady at St. Pierre at 10m and 20m, driven by recruitment of *Pocillopora eydouxi* (Fig. 3). Macroalgal cover was a minor part of the reef habitat at Astove and St. Pierre, but at Assomption there was a rapid increase in macroalgal cover (primarily *Halimeda*) at 10m, and a steady, but less dramatic, increase at 20m following a major decrease in macroalgae at this depth in 2003. Erect dead coral

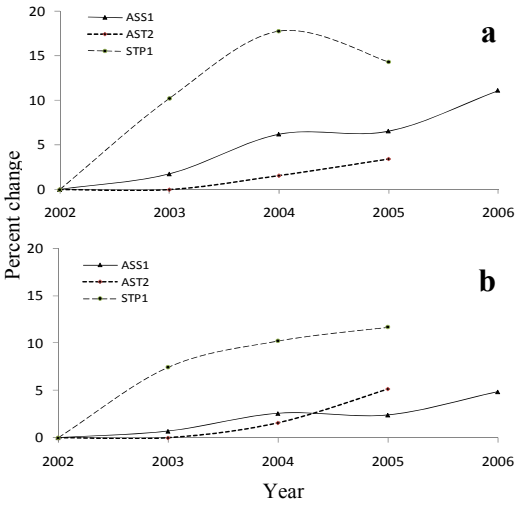

Figure 3. Change in hard coral at Assomption, Astove and St. Pierre (see legend) at a) 10m and b) 20m depth between 2002 and 2006.

formed minimal habitat at Assomption, Astove and St. Pierre at both 10m and 20m, and is generally decreasing.

Fish surveys

The number of species in the annual fish surveys were as follows: Aldabra Atoll – avg. 178, ra. 138-191; Assomption – avg. 90, ra. 88-92; Astove – avg. 88, ra. 83-94; St. Pierre avg. 108, ra. 107-110. At Aldabra the annual means of fish abundances by species, taking into account site differences, showed there were significant differences between sites and years at both 10m and 20m (ANOSIM: Year – 10m P = 0.02, Rho = 0.425; 20m P = 0.03, Rho = 0.316; Sites – 10m P = 0.01, Rho = 0468). However, there were no directional changes following a linear sequence within sites (multiple Figures not shown), with the exception of Site 4 (20m) where there was high annual variability in ten species of schooling, reef-resident planktivores. Site 4 (20m) was no longer significant when these species were removed from the analyses. MDS plots of fish abundances between years at all 10m and 20m sites at Aldabra Atoll (Fig. 4)and single sites at Assomption (Fig. 5), Astove (Fig.

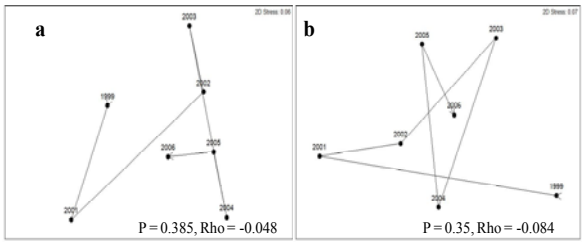

Figure 4. MDS of fish abundance between years for Aldabra atoll (all sites) at a) 10m and b) 20m depth. Line represents trajectory between years and P and Rho are for RELATE seriation analysis.

6) and St Pierre (Fig 7) showed no linear sequence or directional changes over time at either depth (significance levels and stress values shown on graphs; Clarke and Warwick 2001).

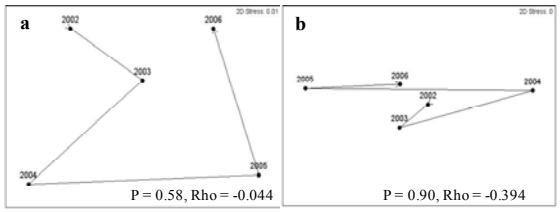

Figure 5. MDS of fish abundance between years for Assomption

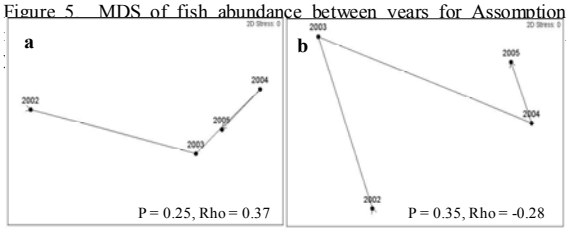

Figure 6. MDS of fish abundance between years for Astove island at a) 10m and b) 20m depth. Line represents trajectory between years and P and Rho are for RELATE seriation analysis.

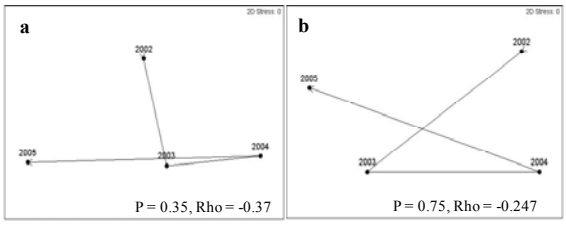

Figure 7. MDS of fish abundance between years for St. Pierre island at a) 10m and b) 20m depth. Line represents trajectory between years and P and Rho are for RELATE seriation analysis.

Discussion

Aldabra Atoll and Assomption, Astove and St. Pierre Islands all suffered massive and extensive bleaching-related losses of live coral habitat in 1998. However, the pre-bleaching reef fish communities at these four locations seem to persist long-term post-bleaching, as demonstrated by the lack of directional changes between years in the MDS plots. Many studies have documented the strong associations between live coral habitat and reef fishes (review in Wilson et al. 2006), as well as the high variability in reef fish communities (review in Holbrook et al. 2006). The responses of reef fishes found in this study leads to an intriguing question of why directional changes in these fish communities have not occurred during eight years following the bleaching event in which there were two location-specific habitat phase-shifts. Change is used here with the caveat of "detectable within the bounds of the methodology used by AMP". There are likely many factors contributing to the lack of change in the assessed reef fish assemblages. We discuss some here in relation to the concept of pushing the ecosystems to their "tipping point", beyond which reduced resilience can lead to change and undesirable phase shifts (Littler and Littler 2007).

The four systems under study were rich in corals and fishes prior to the 1998 bleaching impacts and coral richness could have influenced the apparent continued-stability responses of the reef fish communities to the single-event loss of up to 50% of the live coral habitat. That is, the 1998 event was not a catastrophic-enough level of decline in live coral in these rich systems to trigger change in the basic reef fish assemblages (after Holbrook et al. 2006).

At Aldabra Atoll the rapid habitat phase shift to *Rhytisma* could have ameliorated the loss of hard coral habitat by providing the modest recovery of the living coral on the reef that could enhance stability of the fish assemblage as noted by Holbrook et al. (2006). For *Rhytisma* to be a factor, it would have to provide an ecologically acceptable alternative to important habitat functions affecting the fish community structure that were likely reduced with the loss of hard coral. There is limited information on any direct habitat functions of soft corals for reef fishes, but there is the potential for indirect effects on the composition of reef fish assemblages through biotic interactions between soft corals and other organisms, including hard corals (Syms and Jones 2001). However, *Rhytisma* habitat was a factor only on Aldabra Atoll and not on the three islands that also had no directional changes in their post-bleaching fish communities. Determining if *Rhytisma* habitat had a positive or neutral, or maybe negative, affect on reef fish communities is beyond the scope of this study, leaving the habitat-function role of *Rhytisma* in this situation open for continued speculation.

The framework of erect dead coral provides reef habitat heterogeneity after bleaching disturbance, which is a major factor in reducing changes in the reef fish community (review in Wilson et al. 2006). However, erect dead coral has not registered as a major habitat at Aldabra Atoll or the other three locations, after showing initial decreases and then minimal percentage changes between years. This is more an artifact of the habitat surveys than a true measure of the presence or rate of deterioration of the dead coral framework. The increases in other biotic habitats, such as the growth of *Rhytisma* at Aldabra Atoll, *Halimeda* at Assomption, and encrusting algae at all four locations, have both covered the surface of coral framework and likely added to its' stability and durability. Complex dead coral habitats can provide the habitat functions required by reef fishes with facultative or no relationships with live coral (Bellwood et al. 2006, Holbrook et al. 2006, Graham et al. 2007). Only those fishes with specific dependence on live hard corals will show marked responses to loss of this habitat (Pratchett et al. 2006, Feary et al. 2007) and there were few of these species in the systems surveyed in this study (Buckley et al. 2005).

The trophic plasticity of the fishes on these post-bleaching habitats would be an important factor in the resilience of the fish assemblages over time. If all but the few coral obligate fishes had the ecological versatility to adapt to any changes in prey resource availability caused by the reductions in live hard coral, then these trophic-functional groups of reef fishes would persist even though there was some variability at the species level (see Spalding and Jarvis 2002).

Direct anthropogenic disturbances to reef fishes can have a greater influence on fish assemblages than bleaching-related losses of live coral habitat (McClanahan 2006). Also, these two processes acting in synergy can confound determinations of the natural responses of the reef fishes to coral habitat impacts. Aldabra Atoll was protected from all but minimal anthropogenic disturbances primarily from local subsistence fishing (Pistorius and Taylor 2009) and the pre-bleaching fish assemblage persisted over time. Similar patterns at neighboring unprotected islands suggest any anthropogenic impacts at these locations were below the thresholds affecting local reef-resident species.

The coral habitat in the huge lagoon at Aldabra Atoll received minimal impacts from the 1998 bleaching event (Stobart et al. 2002). This physically protected area is thought to function as a sanctuary for reproductive-age adults and as a nursery area for many reef fishes (AMP, unpublished data). This reproductive potential could provide a steady supply of larval and juvenile fishes for the outer reefs at Aldabra, as well as to islands in the surrounding region. Even at moderate levels, this output could possibly override any bleaching-related loss of local and regional reproductive potential. This is an area of future research by AMP.

The findings here support earlier assessments that the reef fishes at Aldabra Atoll continue to reflect pre-bleaching reef fish community structure, and abundance changes appear to represent the ecology of the "natural" system (Downing et al. 2005, Downing et al. 2006). The pre-bleaching reef fish communities also appear to persist at Assomption, Astove and St. Pierre Islands. It is apparent that the 1998 bleaching impact was not a catastrophic disturbance to these systems. If there was any synergy between the loss of live coral and other post-bleaching biotic or habitat factors, it does not appear to have pushed the ecosystems past their tipping points, initiating directional changes in the associated reef fish assemblages.

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Cozumel Island, México: A disturbance history

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Abstract. This study aims to determine the damage caused by the 2005 hurricanes "Emily" and "Wilma" on the landscape structure of the Parque Nacional Arrecifes de Cozumel (PNAC), México. We conducted samplings at six reefs located in the PNAC during May 2005 and May 2007. At each reef, six 30-m point-intercept transects were run parallel to the coast. The bottom elements that were quantified were coral, sponges, macro algae, coral with recent death, rock and sand. Landscape structure was quantified with the Pielou´s evenness index (J´), and changes were evaluated with non-metric multi-dimensional scaling (NMDS) and the Bray-Curtis similarity coefficient. The results show a significant decrease in the percentage of live cover, and an increase in the percentage of sand and rock. Corals were the most affected group decreasing from 24% to 10% in cover after the two hurricanes; fortunately, cover has increased to 16% by May 2007. Significant differences were found in the landscape evenness, being lower in July 2005($F_{5,210}$= 14.94, P=0.00),; the high similarity of J´ between May 2005 and May 2007 indicates a clear trend of recovery in the reefs. The NMDS show that the two hurricanes affected Cozumel reefs with varying intensity, with "Wilma" having an impact four times higher than "Emily".

Key words: Hurricanes, reefs, algae, Scleractinian coral, sponge, sand, rock.

Introduction

Tropical storms are usually seen as natural disturbances that modify the structure of biotic assemblages in short time periods on tropical reefs (Woodley et al. 1981). However, these phenomena also modify the abiotic components of the landscape and therefore their influence on biodiversity is not just immediate but can last for many years (Michener et al. 1997).

The most developed reefs in Mexico are in the Caribbean (Jordan-Dahlgren 1993). These reefs represent the northernmost part of the Mesoamerican Reef, recognized as one of the most important reef systems in the world (Almada-Villela et al. 2003). In this region, the island of Cozumel represents a key ecological reserve because of the high species richness and the complexity of the ecosystem (Jordán-Dahlgren and Rodríguez-Martínez 2003; Fig. 1). Cozumel reefs are also highly important for the socio-economics of the region, since they attract thousands of divers and snorkelers by its spectacular underwater spots (Spalding 2004).

During 2005, the island of Cozumel suffered the impact of two of the strongest hurricanes in record, "Emily" and "Wilma" (Alvarez - Filip 2007). The first one hit the coast of the Yucatan Peninsula on July 17, with sustained winds of 215 km/h and gusts up to 260 km/h. The eye of the hurricane passed about 5 km from the southern tip of Cozumel, moving in southeasterly direction, so the intensity of the winds diminished to the north of the island. A few months later (October 21 to 24), "Wilma" affected the island. This is the strongest hurricane recorded in the Atlantic Ocean (the tenth globally), with the lowest atmospheric pressure recorded historically in the Western Hemisphere (882 millibars at sea level, Nava-Alvarez 2005). The hurricane eye (63 km in diameter) moved across Cozumel with sustained winds of 220 km/h and gusts of more than 300 km/h, and moved very slowly, at a speed of less than 5 km/h, heading north-northwest (Alvarez-Filip 2007).

The hurricanes not only caused high mortality of benthic species but also moved away enormous volumes of sand and left large areas of bare rock, devoid of colonizers (Alvarez-Filip and Gil 2006).

Considering the strength and extent of the perturbation, the relevance of the reefs, and the need for a systematic evaluation of the effects on the entire ecosystem, our objective was to determine the effects of "Emily" and "Wilma" and the subsequent changes on the landscape structure of the reefs within the limits of the marine protected area of the Parque Nacional Arrecifes de Cozumel (PNAC). We observed a remarkable decline in all the biotic components and a considerably increase on bare rock after the hurricanes. During the 2 following years the reefs started to recovery, however, the general landscape may not be returning to its original state.

Material and Methods

Study area. Cozumel (Fig 1) covers an area of 647.33 km^2, is located approximately 22km from coast of Quintana Roo. The island is occasionally affected by hurricanes in summer and fall (June to November), although August and September are the months with more intense activity. The southwestern coast of the island has been under official protection since 1980; but was in 1996 when Natural Protected Area decree (INE 1998). The Parque Nacional Arrecifes de Cozumel (PNAC), which is the official name, goes from the north end of Paraiso reef (20º 35´ 22 N, 86º 43´ 46 W) to the southernmost tip of the island, and then continues north as far as Punta Chiqueros in the windward side (20º 16´ 11 N, 86º 59´ 26 W; Fig. 1).

Field Work. We conducted seven visits to the area from May 2005 to May 2007. In each period we surveyed a total of six reef (Fig. 1), and a total of 36 transects were done per visit (six at each reef) using SCUBA equipment. and at a depth of 15 m.

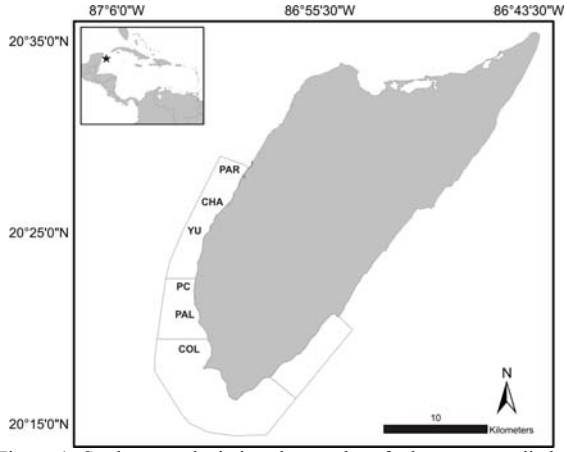

Figure 1: Study area, depicting the coral reefs that were studied at Cozumel Island. PA= Paraiso, CHA= Chanlana´ab, YU= Yucab, PC= Paso del Cedral, PAL= Palancar, COL= Colombia. Continuous line delimits the polygon of the Parque Nacional Arrecifes de Cozumel.

We used the point interception method to evaluate the components of the benthic substrate. The procedure consisted of placing a 30 m-long transect parallel to the coast and recording the type of bottom found directly below points signaled every 25 cm (120 sampling points in total per transect; Almada-Villela et al. 2003), and calculating percentage of bottom cover by each one. Then, the information was systematized and classified in ten major groups: bare rock, sand, dead coral, fleshy macroalgae, sponges, octocorals, hexacorals, turf algae, cyanophytes and coralline algae.

Statistical analysis. Using the percentage of cover of each bottom component, we calculated the Shannon-Wiener diversity index (base 10) and Pielou´s evenness index for each transect, reef and season. These indicators are recognized as among the best to characterize the complexity of the landscape (Jongman et al. 1995; Magurran 2003). Next, the level of change in the reef background among sampling dates was determined with analysis of variance, after confirming homoscedasticity and normality of the data (Zar 1999). Finally, we computed the similarity along landscape compositions in the different samplings with the Bray-Curtis similarity coefficient, and mapped the full information with a non-metric multidimensional scaling (NMDS; Martínez and Adarraga 2001).

Results

Abiotic components. In May 2005 the cover of sand and rock recorded together was 26% in the six sites. During July 2005, when the island received the impact of "Emily", the situation remained very similar, although the amount of rock increased as a result of sediment transport to deeper area. However, in the sampling after "Wilma" and the next one (May 2006) the bare rock was by far the dominant bottom type (~ 40% in average). In the last two visits, the rock decreased its percentage cover to about 25% and remained around that figure to the end of the study. Comparatively the amount of sand was much more stable; it actually decreased after "Wilma" but double its average by the following May (2006), and increased gradually since. Dead coral has been a minor component in the entire study, and had higher values after "Emily". By the last three censuses, dead coral cover was remarkably low, probably because most diseased or damaged colonies were broken and transported away from the reef by the hurricanes.

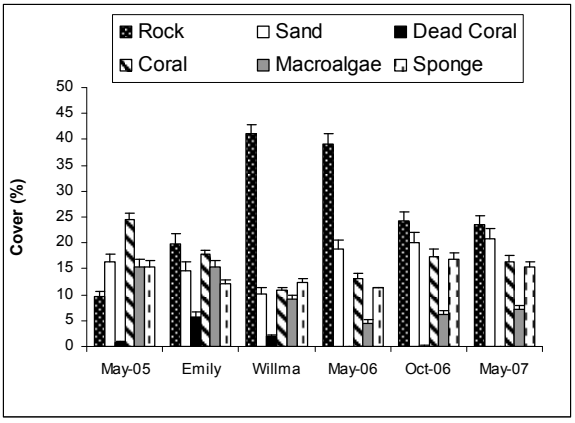

Figure 2: Average cover of the most common bottom types at Cozumel reefs, between May 2005 and May 2007.

Biotic elements. In the case of sponges, their abundance first diminished because of the mass mortality caused by the hurricanes (Alvarez-Filip and Gil 2006), but there was a considerable rebound by May 2007 (Fig. 2). There were significant differences between seasons ($F_{5, 210}$= 41.456, p< 0.0001), caused by the low average found in May 2006, immediately after the hurricanes.

The behavior of the coral abundance at Cozumel was similar to that of the sponges, and we also depicted significant differences between seasons ($F_{5, 210}$= 16.86, p< 0.0001), although in this case were caused by the high cover in May and July 2006. Afterwards the average did not differ (fluctuating between 8% and 12%), an indication of a certain stability and long-term effects of the hurricanes on this group (Fig. 2).

The macroalgae behaved in the same way that corals ($F_{5, 210}$= 22,971, p< 0.0001) and the significative differences separated the higher values from May 2005 and July 2005 (after "Emily"), to the remainder. It is also apparent that the declining trend continued until spring 2006, and from then on the algal cover increased but still is much lower than the original.

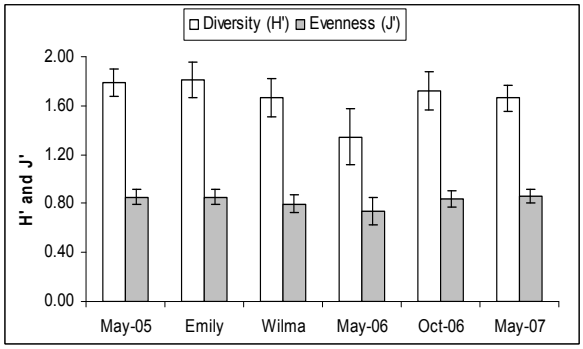

Figure 3: Diversity and evenness of the reef landscape at Cozumel Island between May 2005 and May 2007.

Landscape approach. Diversity and evenness of the landscape components was very stable at Cozumel (Fig. 3), although in both cases a clear and significant decrease occurred in May 2006 from the rest of samplings. Finally, the NMDS (Fig. 4) shows three stages in reef landscape composition between 2005 and 2007: first, the data from May 2005 and the first hurricane ("Emily"), then "Wilma" and the ensuing sampling in spring 2006, and finally the last visits (October 2006 and May 2007).

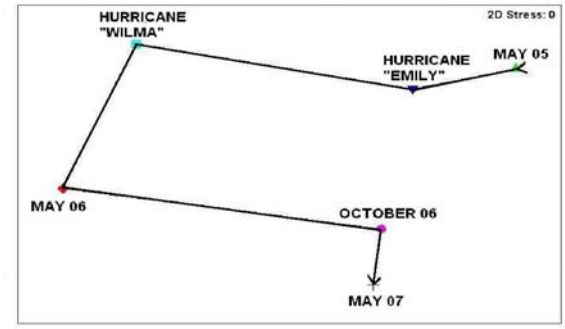

Figure 4: Non metric dimensional scaling comparing the bottom composition at the six sampling seasons (May 2005 to May 2007).

Discussion

This paper is one of the first to analyze the effects of hurricanes from a landscape perspective in México. We consider that this was the correct approach since the passage of hurricanes "Emily" and "Wilma" affected not only the live elements but also the abiotic ones. Then, by taking a more inclusive view we may have a better image of the long-lasting consequences to the composition and function of the system that if only we focus on specific taxa.

As shown by Fig. 2, during the study period we testified a major shift in the proportions of components of the substrate, directly caused by the incidence of two hurricanes in 2005. In general, there was a remarkable increase of the amount of bare rock and a decline in all biotic components, and probably both events were due to the movement of sand across the reef, and its eventual discharge to the slope (Alvarez-Filip and Gil 2006). A similar situation was faced by other locations along the Mexican Caribbean coast, and more specifically at Cancún (McField et al 2008), but the long term results differed remarkably. At Cancún the lack of reefs and limited generation of new sand caused a net deficit and the need to carry new sand to the beach, taken from deep areas, while at Cozumel the coral barrier naturally supported enough material so the amount of sand in the reef soon returned to normality (Fig. 2).

The loss of sand can have important consequences at Cozumel; water clarity diminished a lot after the

hurricanes because of the floating sand grains (LAF personal observations), and that may have altered the metabolism of the zooxanthellae, decreasing in turn the amount of food for corals and the rest of the community, and probably affecting the reproductive success of scleractinians (Chavez et al. 2003; Fabricius 2005). In addition, Alvarez-Filip (2007) indicated that as the large amount of sand moved to the barrier reef and surrounding areas in 2005 left a sizeable area of new substrate, it was to be expected that many new areas were available for colonization. If this is the case, we can expect a high incidence or benthic recruitment in years to come. As the data in Figure 2 show, sponges and corals may have been responding to this opportunity, but nevertheless their slow growth rate forces that the process should take many years (McMurray et al. 2008). This hypothesis checks well with Fenner (1991) observation that after Hurricane "Gilbert", it was expected at least 10 years for recovery of Cozumel reefs.

Many studies have documented changes severe damage to coral colonies and populations due to the behavior of the waves and sea currents during a hurricane (Woodley et al. 1981; Rogers 1993; Kjerfve et al. 1996), and a consequent change in the composition of the biotic components (Done 1992; Bythell et al. 1993; Bahena et al. 2000). At Cozumel, before 2005 were clear differences in coral cover among reefs, but after 2005 we noticed an homogenization of abundance all over the national park (Millet-Encalada et al. 2007). In general this took place because differential mortality concentrated in the two main genres (*Agaricia* and *Porites*), which because of its morphology are much more prone to mechanical damage.

The general trend in the coverage of the sponge was a reduction after 2006 (Fig. 2). This was expected considering fragile structure of these animals that can be easily damaged by the abrasion caused by sediment movement, and to excessively high speeds in water flow (Done 1992; Fenner 1991). However it is important to note that many broken colonies are recovering its size using cellular growth (Stoddart 1974), and probably the cover will increase faster that anticipated by asexual recruitment.

As for the percentage of macroalgae found, this was very variable during the study but fortunately cover was never too high; this means that there is no indication of a phase shift due to the opening of colonization spaces in the reef. Very probably the control of algal proliferation was exerted by urchins but mostly, by herbivorous fishes, which numbers are higher than 2 fishes/m^2 (Díaz-Ruiz and Aguirre León 1993; Lozano Alvarez et al. 2007). Considering this information we support the idea that Cozumel reefs can still be considered as healthy, even when human use is very high (Rioja-Nieto and Sheppard 2008).

In this study we applied diversity and evenness indices (H' and J') to describe the complexity of the reef landscape in the study region. The data showed a clear decrease in May 2006 which reflects the post-hurricane response to two major perturbations, however, both indices rapidly return to the original figure and by the end of the study there was no statistical difference in the indicators. This result may be the best indication of the reef resilience.

Although our data show that Cozumel reefs resisted well the effect of the hurricanes, there are still changes of notice. The NMDS clearly shows that between 2005 and 2007 there have been three distinct stages in reef succession: May to July 2005, late 2005 and early 2006, and the last two samplings (Fig, 3). The fact that the arrow did not return to the original position in the graph remarks that the reefs are showing signs of recovery, but are not "returning" to its original condition; a more accurate view is that the landscape is in dynamic equilibrium, and we need more years to actually confirm what direction is taking.

In conclusion, the effects of two major hurricanes on the landscape diversity of Cozumel reefs were clear, but in a very short time span the animal and plant communities as well as the abiotic environment, seem to be regaining their position. It is probable that if no other perturbation of this caliber occurs, we can assess a complete recovery of the ecosystem. However, the situation may not be so adequate in the near future considering the projected increase in anthropogenic pressures (especially coastal development) for the entire Mexican Caribbean. It is vital to keep a proactive position for management and preview what problems can occur if the number of visitor keeps increasing, and also to continue monitoring and Before –After studies (Michener et al. 1997), fundamental to assess the long-term effects of these perturbations.

Acknowledgement

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Population status of the urchin *Diadema antillarum* in the Florida Keys 25 years after the Caribbean mass mortality

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Abstract. The 1983-84 Caribbean-wide mortality of the urchin *Diadema antillarum* Philippi was followed by a second mortality event in the Florida Keys in 1991. The demise of this once ubiquitous herbivore is one factor contributing to wider Caribbean reef change during the past 25 years. Over an 8-year period from 1999-2007, we examined densities and test sizes of *D. antillarum* at 786 sites from the northern extent of the Florida Reef Tract to the Dry Tortugas, including two National Parks and the Florida Keys National Marine Sanctuary. Visual surveys along belt transects were used to enumerate individuals and test sizes in a two-stage stratified random sampling design that incorporated cross-shelf benthic habitats, geographic regions, and no-fishing management zones. While pre-1983 densities were as high as 5 individuals/m^2, surveys since 1999 from < 1 m to 27 m depth show that current densities are still well below 1 individual/m^2. During seven different annual sampling periods, the maximum site-level density was only 0.33 individuals/m^2, with the highest densities of larger (> 5 cm test diameter) individuals reported from only a few locations. The relative contributions of larval survivorship, predation, suitable recruitment sites, and reduced fertilization success to *Diadema* recovery are still largely unknown.

Key words: Benthic, *Diadema*, marine protected area, stratified sampling, urchin.

Introduction

The 1983-84 Caribbean-wide mass mortality of the long-spined sea urchin *Diadema antillarum* represents one of the most spatially expansive and prolonged disturbances to reef ecosystems in the region (Carpenter 1988; Lessios 1988, 2005). Prior to the mass mortality event, *D. antillarum* attained high (>20 per m^2) densities in many Caribbean reef areas (Lessios 1988), but urchin abundances declined by several orders of magnitude and have remained in this state over two decades later (Lessios 2005; Weil et al. 2005; Debrot and Nagelkerken 2006). Together with physical impacts from storms, coral disease outbreaks, and severe bleaching episodes (Gardner et al. 2003), the reduction in urchin densities has had severe consequences in terms of coral-algal dominance patterns (Carpenter 1988; Lessios 1988).

In the Florida Keys, the few historical data available prior to 1983-84 indicate that *Diadema antillarum* densities were lower (up to 4-5 per m^2) (Kier and Grant 1965; Bauer 1976, 1980). However, still a general trend of increased algal cover was apparent after the urchin mortality at several Florida Keys reefs (Jaap et al. 1988; Porter and Meier 1992). Seven years after the 1983-84 event, a second disease event in the Florida Keys, after initially modest recovery to 0.30-0.58 individuals/m^2, once again depressed *D. antillarum* densities to < 0.01 individuals/m^2 (Forcucci 1994). Large-scale surveys of urchin densities across the south Florida during 1999-2001 confirmed this pattern of poor recovery (Chiappone et al. 2001, 2002).

Since the mass mortality, limited to moderate recovery in *Diadema antillarum* populations has been reported in some Caribbean reef areas (Lessios 2005; Carpenter and Edmunds 2006; Debrot and Nagelkerken 2006), but this has not occurred in the Florida Keys (Chiappone et al. 2002; Lazar et al. 2005). It is anticipated that *D. antillarum* recovery will help to promote coral recruitment and a return to pre-mortality baseline reef conditions (Carpenter and Edmunds 2006; Macia et al. 2007; Myhre and Acevedo-Gutierrez 2007). There is particular interest in the spatial and temporal patterns of recovery in the Florida Keys, as there are expectations that urchin recovery will perhaps help to reverse the trend in seaweed expansion and concurrent declines in reef-building corals. In addition, the slow and incomplete recovery of this urchin raises the question of factors limiting population recovery (Miller et al. in press).

Since 1999, we have conducted intermittent, large-scale surveys of urchin density and size structure in a diversity of habitats across the south Florida shelf encompassing hundreds of sites. This short communication describes the population status of *Diadema antillarum* as of 2007 along ~200 km of the

Florida Reef Tract, but also provides a temporal comparison of population densities and size structure since 1999 in both the Florida Keys and Dry Tortugas regions. These results are a follow-up to previous reports on the population status of urchins in the Florida Keys (Chiappone et al. 2001, 2002) and are part of an ongoing assessment and monitoring program to evaluate large-scale ecological patterns in community structure and the responses of small reef areas to protection from fishing pressure (Miller et al. 2002).

Material and Methods

The Florida Keys are an archipelago of limestone islands stretching more than 360 km from Key Biscayne to the Dry Tortugas. Along the seaward edge of the south Florida shelf is the reef tract, a semi-continuous series of offshore bank-barrier reefs interspersed with sand, rubble, and low-relief hard-bottom (Shinn et al. 1989). Between the islands of the Florida Keys and the reef tract is Hawk Channel, a V-shaped basin (5-12 m depth) dominated by sand, seagrasses, and patch reefs (FDEP 1998). Coral reef distribution and community structure in the Florida Keys reflect exchange processes between Florida Bay and the Atlantic Ocean affected by the size and orientation of the Pleistocene islands and the proximity of the Florida Current to the platform margin (Shinn et al. 1989).

During 1999-2007, a series of research expeditions were carried out to quantify, among other variables, urchin density and size distribution patterns across the south Florida shelf. A two-stage, stratified random sampling approach was used to derive mean density estimates for urchins at multiple spatial scales throughout the Florida Keys, following similar procedures outlined in Cochran (1977) and modified for our coral reef benthic surveys (Miller et al. 2002; Smith et al. in press). Surveys were carried out in the Florida Keys, excluding the Dry Tortugas, in 1999-2001 and 2005-07, and in the Dry Tortugas during 2000 and 2006 (Figs. 1-2). The sampling domain was partitioned by habitat type using available bathymetry and benthic mapping data (FDEP 1998). The habitat classification scheme accounted for features that correlate with benthic fauna distributions, including cross-shelf position, topographic complexity, and the proportion of sand interspersed among hard-bottom structures. Additional stratification variables included regional location and management zones, the results of which are reported elsewhere. Map resolution was such that the survey domain was divided into a grid with individual cells of size 200 m by 200 m (40,000 m^2) that defined unique habitat classes. Table 1 provides the classification system used during the 2007 surveys at 235 sites in the Florida Keys.

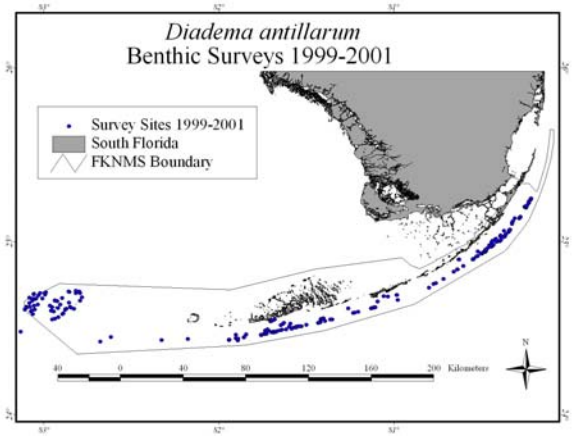

Figure 1: *Diadema* urchin survey sites in the Florida Keys National Marine Sanctuary (FKNMS) sampled during 1999-2001.

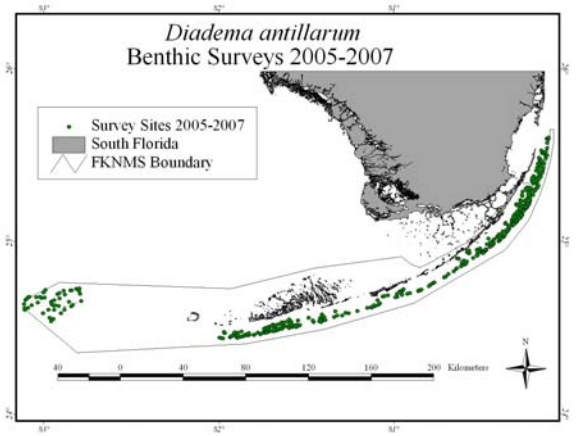

Figure 2: *Diadema* urchin survey sites in the Florida Keys National Marine Sanctuary (FKNMS) sampled during 2005-07.

During each sampling mission, four random sampling points were generated in a GIS and were located in the field using a differential global positional receiver. During 1999-2001, paired 10 m or 25 m transects, depending on the site area, were deployed at each of the four sampling points. A 0.4 m scale bar was used to sample along both transect sides for the number and test diameter of urchins. During 2005-07, four 15-m x 1 m belt transects were sampled per site. Urchin density calculations were calculated for each site and for each stratum, consisting of combinations of habitat, regional location, and management zone. Data reported herein emphasize habitat-related patterns in density from the 2007 surveys, but also temporal comparisons between 1999-2001 and 2005-2007. Statistical comparisons of mean densities were accomplished by computing confidence intervals (CI) based on the equation: CI = mean $\pm$ $t_{[\alpha,\ df]}$ *standard error. Standard errors were estimated by the two-stage, stratified random sampling design (Cochran 1977) and confidence intervals were adjusted for multiple comparisons

using the Bonferroni procedure. The experiment-wise error rate was held at $\alpha = 0.05$ and the comparison-wise error rate was adjusted based on the number of multiple comparisons as follows: comparison-wise error rate = α/c, where $c = k(k-1)/2$ and k = number of categories (e.g. habitat classes).

Table 1: Characteristics of *Diadema* urchin survey sites in the Florida Keys sampled during 2007. Available sites reflect the number of 200 m x 200 m cells containing particular habitat types based upon FDEP (1998) mapping data

Habitat	Depth (m)	Sites (% effort)	Sites available
Mid-channel patch reef (MPR)	0.9-2.7	36 (15.3)	3,532
Offshore patch reef (OPR)	2.1-14.6	42 (17.9)	1,170
Hard-bottom/rubble/sand matrix	2.7-5.8	4 (1.7)	79
Shallow hard-bottom (LHBS)	2.7-7.0	25 (10.6)	972
Inner line spur and groove (IRT)	1.5-6.1	8 (3.4)	87
High-relief spur and groove (HSG)	0.6-9.4	51 (21.7)	238
Deeper hard-bottom (LHBD)	6.7-13.7	15 (6.4)	1,962
Patchy hard-bottom (PHBD)	4.6-11.3	21 (8.9)	956
Low-relief spur and groove (LSG)	7.6-16.2	33 (14.0)	2,825
Sampling Design Total	0.6-14.6	235 (100)	11,821

Results

Results presented herein detail *Diadema antillarum* population density and size structure based upon 2007 surveys, as well as temporal comparisons encompassing 1999 through 2007. Surveys of 235 sites from the northern Florida Reef Tract to SW of Key West during 2007 yielded relatively low densities, but slightly larger test sizes compared to previous sampling periods. A total of 299 individuals were recorded, with individuals distributed among all nine habitats sampled. The maximum site-level density was 0.267 individuals/m^2 from an upper Florida Keys offshore patch reef. Relative to previous years, an appreciable increase was apparent in the number of sites where *D. antillarum* was found and a general trend towards larger test sizes. Densities were greatest on offshore patch reef and high-relief spur and groove reefs (Fig. 3). However, habitat-level densities were so low that, despite the relatively large sample size, the only significant difference detected was between the offshore patch reef and deeper hard-bottom habitats ($P < 0.0018$, Bonferroni-adjusted α). Of the 299 *D. antillarum* recorded, test sizes ranged from 0.3-10.0 cm, with a mean ± 1 SE test size of 3.6 ± 0.1 cm. The test size distribution, in contrast to previous years, included a relatively large proportion (~33%) of individuals > 5 cm TD (Fig. 3).

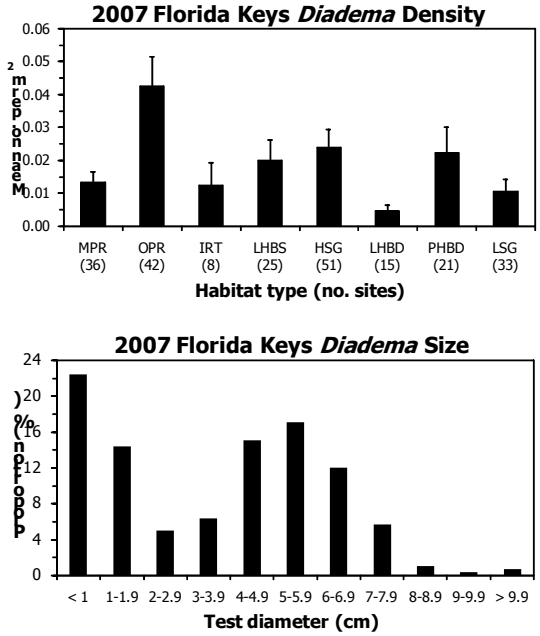

Figure 3: Mean *Diadema antillarum* densities by habitat (top) and test size distribution (bottom) from surveys of 299 individuals at 235 Florida Keys sites during 2007. Error bars are +1 SE and numbers in parentheses on the x-axis (top) are the number of sites sampled in each habitat. See Table 1 for habitat abbreviations.

Table 2 summarizes the temporal in *Diadema antillarum* density and size structure during two time periods in the Florida Keys and Dry Tortugas. In the Florida Keys, a greater proportion of sites yielded no *D. antillarum* in 1999-2001 (75%) compared to 2005-07 (62%). However, very few sites in either time period (≤ 1%) yielded densities greater than 0.2 individuals/m^2. The range in test size, the maximum test size, and the mean test size has increased since 1999-2001. The largest apparent changes are the ~42% increase in mean test size and the proportion of urchins larger than 5 cm TD in 2005-07 (32%) compared to 1999-2001 (9%).

For the Dry Tortugas region, including both shallower (< 15 m) habitats within the National Park and deeper (> 15 m) habitats on the Tortugas Bank, there are temporal similarities in density distribution, but differences in size distribution with the rest of the Florida Keys (Table 2). A greater proportion of sites yielded no individuals in 2000 (59%) compared to 2006 (44%), and very few sites in either time period (< 5%) yielded densities greater than 0.2 individuals/m^2. In terms of size structure, the most notable differences between the two time periods were the dramatic (58%) decrease in mean test size, as well as 28% change in the proportion of

individuals larger than 5 cm TD, a pattern opposite to that documented in the rest of the study area.

Table 2: Temporal summary of *Diadema antillarum* density and size structure in the Florida Keys and Dry Tortugas regions during 1999-2001 and 2005-07. Site-level densities are the number (proportion) of sites with a given mean density value

Study area/metric	1999-2001	2005-07
Florida Keys		
No. sites	211	495
No. habitats	9	10
Depth range (m)	1.2-14.0	0.6-27.0
Area sampled (m^2)	16,400	29,700
Site-level density		
Absent	158 (74.9)	307 (62.0)
0.01-0.05 per m^2	50 (23.7)	153 (30.9)
0.06-0.10 per m^2	1 (0.5)	24 (4.8)
0.11-0.15 per m^2	0	6 (1.2)
0.16-0.20 per m^2	2 (0.9)	3 (0.6)
0.21-0.25 per m^2	0	1 (0.2)
0.26-0.30 per m^2	0	1 (0.2)
0.31-0.35 per m^2	0	0
0.36-0.40 per m^2	0	0
0.41-0.45 per m^2	0	0
Size structure		
No. individuals	88	474
Range in test size (cm)	0.3-6.7	0.3-10.0
Mean test size (cm)	2.4	3.4
SE test size (cm)	0.2	0.1
% of urchins > 5 cm TD	9.1	32.4
Dry Tortugas		
No. sites	34	46
No. habitats	9	11
Depth range (m)	4.0-24.1	2.7-26.1
Area sampled (m^2)	1,360	2,760
Site-level density		
Absent	20 (58.8)	20 (43.5)
0.01-0.05 per m^2	11 (32.4)	19 (41.3)
0.06-0.10 per m^2	1 (2.9)	4 (8.7)
0.11-0.15 per m^2	0	0
0.16-0.20 per m^2	1 (2.9)	1 (2.2)
0.21-0.25 per m^2	0	0
0.26-0.30 per m^2	0	0
0.31-0.35 per m^2	1 (2.9)	0
0.36-0.40 per m^2	0	2 (4.3)
0.41-0.45 per m^2	0	0
Size structure		
No. individuals	34	98
Range in test size (cm)	0.9-9.0	0.3-7.8
Mean test size (cm)	4.3	1.8
SE test size (cm)	0.4	0.2
% of urchins > 5 cm TD	44.1	16.3

Discussion

Large-scale surveys encompassing hundreds of sites across the south Florida shelf since 1999 indicate that the *Diadema antillarum* population exists at densities well below values reported before the two mortality events in 1983-84 and 1991 (Kier and Grant 1965; Bauer 1980; Forcucci 1994). For the Florida Keys there has been an increase the proportion of sites with *D. antillarum* present, as well an increase in mean test size, with a greater proportion of larger individuals in the population. Earlier reports and recent observations indicate that other urchin species show density and habitat distribution patterns similar to pre-1983 observations, indicating that other species have not compensated for the loss of *D. antillarum* (Chiappone et al. 2002).

In the Dry Tortugas, the past six years have witnessed a decline in average size, possibly indicative of a recruitment event, perhaps in 2005, or poor survivorship to larger sizes, or both. It is noteworthy that Dry Tortugas surveys conducted in 2006 followed a very active tropical cyclone season in 2005, where several named storms passed directly over or in close proximity to the Dry Tortugas. In areas with relatively high (> 0.1 individuals/m^2) and larger (> 5 cm TD) *Diadema antillarum*, there are obvious effects of grazing on the substratum, particularly the removal of turf and macroalgae and exposure of reef rock (Chiappone et al. 2001). It remains unclear at this time whether increasing urchin densities and sizes will lead to other changes such as increased coral recruitment.

The very slow and prolonged recovery in the Florida Keys, especially compared to several recent studies in other Caribbean reef areas, raises several questions pertaining to the possible factors inhibiting recovery. Lessios (1988) and others have discussed the merits of hypotheses concerning population recovery, among them poor larval survivorship, lack of adult conspecifics and hence protection from predators, suitable recruitment sites, and inter-specific competition. The sources of urchin larvae to the south Florida shelf are not known, but may include both local and regional sources (Lee et al. 1994). Nonetheless, it is apparent that *D. antillarum* have continually recruited to benthic habitats, even after the 1991 mortality event (Chiappone et al. 2002). A recent study of *D. antillarum* larval settlement rates in the Florida Keys, however, indicate that low larval supply may be one factor limiting recovery (Miller et al. in press). The predominance of relatively small test sizes from 1999-2007 indicate post-settlement recruits have poor survivorship, perhaps due to predation pressure or physical disturbance from storms. Because *D. antillarum* was historically significant as a grazer it will be important to monitor its change in abundance and size structure over time, as well as the possible effects on benthic community structure in the Florida Keys.

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Coral Reef Monitoring for the Organization of Eastern Caribbean States (OECS) and Tobago

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Abstract. The objective of this project was to expand the coral reef monitoring programme started under the CPACC (Caribbean Planning for Global Climate Change) Project to the countries of the Eastern Caribbean. The Centre for Marine Sciences (CMS) of the University of the West Indies (UWI) Jamaica was contracted by the MACC (Mainstreaming Adaptation to Climate Change) Project to oversee this process. Sixteen participants from seven Eastern Caribbean countries (Antigua & Barbuda, Dominica, Grenada, St Kitts & Nevis, Saint Lucia, St Vincent & the Grenadines and Tobago) were trained in the coral reef video monitoring protocol developed under CPACC in Saint Lucia during September 2007. Following the training, each country selected a monitoring site and monitoring was conducted (during the period September to April 2008) at these sites under the supervision of resources persons from the CMS. The videotapes were processed and analyzed at the CMS. This paper will discuss the results of the monitoring exercise for each of the countries and will highlight the challenges encountered in establishing a regional monitoring programme that meets the needs of the participating countries

Key words: Eastern Caribbean, coral monitoring, video monitoring, climate change

Introduction

The Caribbean Community Climate Change Centre (CCCCC) was established by regional governments to coordinate the Caribbean's response to climate change. One of the projects being executed by the CCCCC is the Mainstreaming Adaptation to Climate Change (MACC) Project which aims to mainstream adaptation to climate change into national development planning through technical support and capacity building. Included in the capacity building programme was the strengthening of the climate and coral reef monitoring networks in the region.

The Centre for Marine Sciences (CMS) under a Memorandum of Understanding with the CCCCC for MACC Project was tasked with leading the coordination and expansion of the regional coral reef monitoring programme to the OECS (Antigua & Barbuda, Dominica, Grenada, St Kitts & Nevis, Saint Lucia, St Vincent & the Grenadines) and Tobago (Fig. 1). This expansion follows up on the recommendations made at the conclusion of the Caribbean Planning for Adaptation to Global Climate Change (CPACC) project based on pilot studies carried out in The Bahamas Belize and Jamaica (Lawrence & Edwards, 2001).

This monitoring programme was implemented by providing technical support in the areas of training, monitoring, data analysis and report preparation. The data collected and reported on in this study is intended to represent the start of a coral reef monitoring programme for the OECS and Tobago which will assist in documenting the changes taking place on the coral reefs over time and will attempt to determine the reasons for the observed changes.

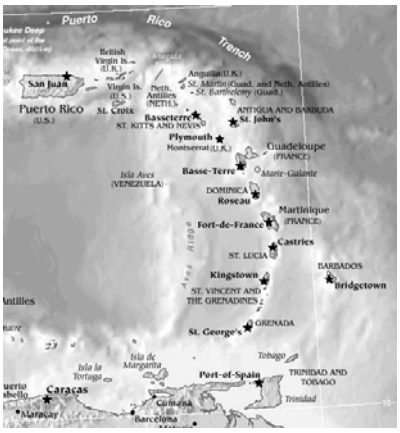

Figure 1: Map of the Eastern Caribbean showing the location of the seven countries (Antigua & Barbuda, Dominica, Grenada, St Kitts & Nevis, Saint Lucia, St Vincent & the Grenadines and Tobago) took part in the coral reef monitoring programme.

Methodology

Training

Training took place during the period September 10-13, 2007 in St Lucia and involved 16 participants from 7 countries. The participants were trained in the CPACC video monitoring protocol which involved site selection,

video monitoring, benthic substrate identification, data processing, data analysis and report preparation (Woodley 1999; Miller 2000; Miller and Roger 2002). At the end of the training workshop one set of video monitoring equipment, provided by the MACC project, was handed over to each of participating countries (Creary 2007).

Monitoring

Following the training workshop the participants returned to their respect countries and selected coral reef areas for monitoring (Operational Area) based on the guidelines outlined in the site selection protocol (Woodley, 1999) which took into consideration the use to which the data would be put, the peculiarities of the area and logistical constraints. Each country was required to establish 20 permanent transects, each 20m long, within the Operational Area selected.

The video monitoring method used was based on the Aronson method that was adapted by Jeff Miller (US GS Biological Division, USVI) for Component 5 of the CPACC project - *Coral Reef Monitoring for Climate Change Impact* (Miller 2000) and with additional modifications included in this monitoring exercise (Miller and Rogers 2002).

A resource person from the CMS led the local teams on the monitoring exercises. The high resolution video cameras provided for each country were used to video tape the individual 20m transects. Approximately 20 transects were recorded for each country. Monitoring was carried out during the period September to November 2007 for all the countries except for Antigua. Field works was started in Antigua during October 2007 (four transects were video taped) but was not completed due to several delays resulting from technical difficulties and poor weather conditions. Because of the long delay in completing the monitoring the entire exercise was repeated in April 2008. Both sets of data are presented in this paper. The monitoring dates along with the locations of the Operational Areas and monitoring sites are provided in Table 1.

Data processing and analysis

The videotapes were catalogued and delivered to the Caribbean Coastal Data Centre (CCDC) of the CMS for processing and analysis. A Sony Mini DV player, connected to a computer and using the Pinnacle Studio™ software, was used to capture non-overlapping adjacent images from the video tapes. The CPCe (Coral Point Count with Excel) programme (Kohler & Gill 2006) was used to randomly overlay 10 points on each image from which the benthic species or substrate category lying under each point was identified. Codes were entered directly into the associate Microsoft Excel spreadsheet which automatically generated statistical parameter for each species/substrate category (relative abundance, mean, standard deviation, standard error and the Shannon–Weaver diversity index).

Country /Dates	Operational Area/ Monitoring Site(s)
Antigua & Barbuda Oct 16, 2007 April 9-11, 2008	Little Bird Island
Dominica Nov 13- 15, 2007	Soufriere/Scott's Head Marine Reserve (SSMR) • Soufriere Pinnacle • Danglebens • Point Guignard • Champagne
Grenada Oct 31-Nov 2, 2007	Grand Anse reef system • Boss Reef • Middle Boss Reef • Bottom Boss Reef • Northern Exposure
St Kitts & Nevis Nov 4-9, 2007	Sandy Point • Paradise Reef
Saint Lucia Sep 12-14, 2007	Soufriere Marine Management Area (SMMA) • Turtle Reef • Anse Chastanet Reef, • Grand Caille, • Coral Gardens, • Malgretoute
St Vincent & the Grenadines Oct 23-24, 2007	Castle Bay
Tobago Oct 16-17, 2007	Speyside, Tobago • Japanese Gardens • Angel Reef

Table 1: Monitoring dates, Operational Areas and monitoring sites

Results

The results of the monitoring programme (Fig. 2 and 3) shows that the sites selected in each country had varying levels of coral cover ranging from 3.8% in Antigua (2008) to 29.2% in St Vincent & Grenadines. Dominica, Grenada, St Kitts and Saint Lucia had coral cover at about 10% while Tobago had 15.9%. Gorgonians were not very abundant in all the islands ranging from 1% to 3.6% with the exception of Tobago which had 16.8% cover. Sponges were also found in the highest abundance in Tobago with 21.2%, St Vincent had a little over half that amount with 12.1%, while St Kitts and Saint Lucia had 7.5% and 7.1% respectively. The other three countries had less than 4% gorgonians. The zoanthids represented a very small proportion of the benthic cover in all the countries ranging from 0% to 0.9%. Also not well represented were the coralline algae which ranged from 0% to 0.9% for all countries except Tobago which had a higher cover of 2.1%. Disease corals were observed in limited amounts in Antigua (2007), Dominica, St Kitts and St Vincent.

It was observed that for all the countries there was a high level of macroalgae combined with dead coral and

algae found on the reefs with Antigua (2007) having a combined value of 56.2%, Dominica having 66.6%, Grenada with the highest at 84.2% and St Kitts at 76.0%. Not unexpected was that Tobago (25.9%) and St Vincent (39.6%) exhibited the lowest levels of macroalgae combine with dead coral and algae, while Antigua – 2008 (44.9%) and Saint Lucia (44.4%) had intermediate levels.

During the monitoring exercise a total of 41 coral species were observed in addition to other unidentified coral species and coral juveniles. The most widely distributed species were *Diploria strigosa*, *Montastraea annularis, M. cavernosa, Porites astreoides* and *Siderastrea siderea,* which were found in all countries and *Agaricia agaricites, Meandrina meandrites, Millipora complanata* and *P. porities* which occurred in six of the seven countries. Just under half (18) of the species identified were found in only one or two countries, with the remaining 15 species occurring in 3-5 countries.

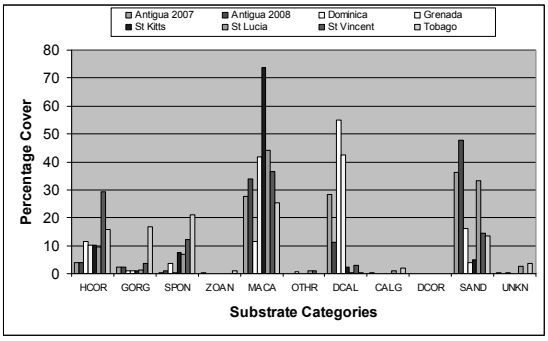

Figure 2: Graph illustrating the mean percentage cover for the different substrate categories found at the coral reefs sites in Antigua, Dominica, Grenada, St Kitts, Saint Lucia, St Vincent and Tobago monitored during the period September 2007 to April 2008. (Substrate categories: HCOR - Hard coral; GORG - Gorgonians; SPON – Sponge; ZOAN – Zoanthids; MACA– Macroalgae; OTHR – Other, live; DCAL - Dead coral with algae; CALG – Coralline algae; DCOR– Diseased coral; SAND – Sand, rubble, rock and boulder; UNKN – Unknown.)

Fig. 3 provides a summary of the hard coral data collected during this study. In Antigua the site selected for monitoring had the lowest percentage cover (4% and 3.8%) when compared to the other islands and also the lowest number of coral species (9 and 13). St Vincent on the other had the highest percentage cover (29.2%) and the highest number (29) of species.

Discussion

The coral reef monitoring programme was successfully implemented in seven countries in the Eastern Caribbean and plans are currently being formulated to conduct the second round of monitoring.

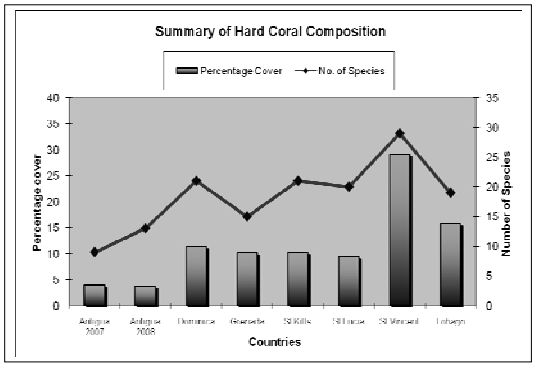

Figure 3: Graph summarising the hard coral composition (percentage cover and number of species) at the monitoring sites in Antigua, Dominica, Grenada, St Kitts, Saint Lucia, St Vincent and Tobago for the period September 2007 to April 2008.

The reefs in the Eastern Caribbean have been exposed to a variety of local (eg pollution and sedimentation from run offs) and regional (eg increase sea surface temperatures) threats, the impacts of which are different for each country. This monitoring programme provides the opportunity to establish a long term programme to assess changes in reef health over the long term. For some countries this monitoring programme would form the basis of the comprehensive reef health assessment while for others the data provided would support existing programmes.

In Antigua, although *Acropora palmata* beds were previously reported to be extensive at Little Bird Island in Antigua no colonies were observed during this assessment and the area appeared to have experienced significant disturbance which supports the observations of Bouchon et al (2004) particularly with respect to the low hard coral (4%) and high algal cover.

In the case of Dominica, it was felt that the 11.6% coral cover and the 21 hard coral species identified were not indicative of the full coral biodiversity within the SSMR (per com Fisheries Department Representative). Bouchon et al (2004) describe the reefs in Dominica as having "high hard and soft coral abundance, very low algal cover, no observable disease, no bleaching". The results from the four sites monitored did not reflect this therefore work is needed for a more accurate assessment of reef health in Dominica.

The Fisheries Department of Granada has proposed a system of marine protected areas for Grenada (Burke & Maidens, 2004) and it is expected that the results from this monitoring exercise will be used in conjunction with previously collected data to support this proposal.

Paradise Reefs, the monitoring site selected in St Kitts is located within the designated National Marine Park at Sandy Point and features giant basket sponges as part of the coral assemblage. Although this area is well used

by divers, its designation as a protected area prevents boats from anchoring and damaging the reefs.

The fringing reef communities along parts of the west coast of St Lucia are under pressure from tourism and coastal development (Burke and Maidens 2004). The SMMA has a comprehensive monitoring programme which provides data on a variety of coastal and marine parameters and the data collected from this monitoring exercise will be incorporated into their database.

For St Vincent & the Grenadines the monitoring site selected at Castle Bay had the highest coral cover and number of coral species despite its close proximity to the capital, Kingstown, and the impacts of runoff and discharges from the nearby rivers.

For Tobago it was felt that the results of the monitoring did not reflected the results of previous studies using photo quadrat surveys carried out in the area by Laydoo (1985) (per com IMA Representative). The main difference cited were the number of coral species found and the actual species identified. A more detailed evaluation of the studies, along with any other additional data that might be available, needs to be carried out before a conclusion can be drawn about these results.

There were a number of challenges faced in the implementation of this monitoring programme which have also served as a learning experience for all those participating in the effort. The most significant challenge was that of adequate numbers of personnel to carry out the preparatory work (site selection, marking of transects), monitoring and data analysis. This was partially overcome by having resources persons from the CMS leading the monitoring in each country and having the videotapes processed and analyzed by the CMS. More time was required for the training of the participants especially with respect to the data processing and analysis component to facilitate the carrying our of in-country analysis. There were also some logistical (e.g. boat availability, access to sites) and equipments problems (e.g. fogging and flooding of camera housings) and the issue of weather played a significant part in the delays experienced in completing the monitoring especially for Antigua.

Recommendations

These recommendations are based on the experience of this first monitoring exercise and should be considered for incorporation in the overall monitoring programme.

Representatives from the participating countries should together review and discuss the technical and logistical challenges of this programme with a view to refining the monitoring process. A review of the training should also be carried with the persons who participated in the training workshop to fine-tune and clarify the video monitoring protocol with specific emphasis on the data processing and analysis components. Training should also be provided to additional persons in the countries that request this, focusing on their specific areas of need. Proposed solutions should then be incorporated into the program for future monitoring.

Each country should review the site selected to ensure, based on the experience of the first monitoring exercise, that these sites are representative of the area being studied and are suitable for the long term monitoring programme. Once suitable sites have been decided on, permanent transects should be established where these have not been done to ensure that the same transects are monitoring over the long term. These sites should be geo-referenced.

There should be an increased focus on in-country coral species identification and data analysis particularly by the persons knowledgeable about the specific monitoring sites. This is presently carried out by the CMS, UWI in Jamaica. Each country should gather all other relevant data related to the site selected on anthropogenic issues such as pollution, over-fishing, sedimentation, turbidity and physical damage, which interact with climate factors and affect the status of the coral reefs.

Provision in the programme should be made carry out monitoring during coral bleaching invents to determine the extent of bleaching and the rate and nature of recovery. The CREWS network in the Caribbean provides the information which allows predictions of potential bleaching events. Closely associated to coral bleaching are the increase incidences of coral diseases which are now being linked indirectly to increased sea surface temperatures. Provision should also be made to include the monitoring of carol diseases if they occur. Where possible, other indicators used for assessing and monitoring coral reef status should be incorporated in the monitoring programme, such as but not limited to, coral recruitment, coral size distribution, fish species and abundance, abundance of herbivores and physical parameters.

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Ecological patterns and status of the reefs of Sudan

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Abstract. Sudan borders the western shore of the Red Sea, a sea recognised as being of global importance for marine biodiversity. A survey carried out during October / November 2007 provided the opportunity to assess the current status of the reefs, many of which have never previously been surveyed. Within the Sudanese Red Sea there is a pronounced biogeographical transition from reef communities that are characteristic of the northern Red Sea to those more representative of the southern Red Sea. Recreational and extractive use of resources is still moderately low however the condition of the reefs and the abundance of resources is highly variable. There are low abundances of several key families of commercial fin fish (particularly groupers and larger snappers) and invertebrate groups (particularly sea cucumbers and larger gastropod molluscs). There is also a conspicuous absence of sea urchins (*Diadema* spp. and *Echinometra* spp.) from many sites. The reefs of Sudan were impacted by previous coral bleaching events, although the extent of mortality was influenced by variability in community composition and local environmental conditions. The biogeographic trends within Sudanese waters and the variability in resource abundance and reef condition all have important implications for management planning.

Key words: Red Sea, sea urchins, coral bleaching

Introduction

The Sudanese Red Sea supports an extensive near-continuous fringing reef, a complex offshore barrier reef system, and numerous uninhabited islands. While some of the more accessible reefs, such as those in the vicinity of Port Sudan and Sanganeb Atoll, have previously been the subject of research (Head 1980, Merger & Schumacher 1985, Edwards & Head 1987, Reinicke et al. 2003), and others are renowned tourism dive sites (e.g. Sha'ab Rumi), many of the reefs have never previously been surveyed. More recent survey efforts focused on the establishment of two marine parks at Dungonab Bay and Mukawwar Island National Park (DBNP) (surveyed in 2002 and 2006) and Sanganeb Atoll Marine National Park (SMNP) (surveyed in 2006) (Fig. 1) improved the understanding about the current status of the reefal communities within these areas (Kemp et al. 2002, PERSGA/GEF 2004, APF 2006).

In 2007 a more geographically comprehensive habitat and biodiversity baseline survey was carried out on some of the un-surveyed reefs in the Sudanese Red Sea. The survey was part of the first phase of a larger programme which aims to link poverty alleviation and the protection of the environment of the Sudanese Red Sea through the implementation of Integrated Coastal Zone Management (ICZM). The ICZM programme, led by Equipe Cousteau, and is part funded by the European Commission in co-operation with a consortium of partners including the Higher Council for Environment and Natural Resources (HCENR) of the Red Sea State of Sudan, UNESCO, and PERSGA (The Regional Organization for the Conservation of the Environment of the Red Sea and Gulf of Aden). This paper presents the preliminary results from the baseline survey that will be used to assist the Government of Sudan to manage the anticipated developments in the coastal region.

Methods

During a four week boat based survey (October—November 2007) on board the MSY Elegante a survey team collected data on the habitats and biodiversity associated with coral reefs along the Sudanese Red Sea coast. The survey area spanned approximately 195 km of coast north to south, and extended approximately 75 km west to east, across 2° of Latitude (between 20°28'N and 18°45'N) and 1° Longitude (between 37°11'E and 38°10'E).

A combination of survey methods was adopted similar to those used elsewhere in the Red Sea and Gulf of Aden region to characterize and map the distribution and status of coastal and marine habitats and associated species assemblages. This paper considers the results of the detailed transect based surveys that were completed at 40 sites along the Sudanese coast. At each of these sites a team of surveyors completed a modified version of the Reef

Check survey protocol which included: (i) video transects to characterize the benthic cover (4 replicate 20 m transects); (ii) timed swims to record the diversity of hard coral species; (iii) belt transects to assess the abundance of key macro-invertebrates (4 replicate 20 m by 2m wide belt transects) and (iv) belt transects using an underwater visual census (UVC) method to record abundance estimates and / or species presence / absence data on indicator fish groups and fisheries groups (4 replicate 5 m belt transect counts, and timed swims). Data collected included: (i) Abundance of butterflyfishes and angelfishes (all individuals identified to species level), (ii) Abundance of groupers, snappers, emperors, parrotfish, (iii) Abundance of Napoleon wrasse and moray eels, and, (iv) Species richness of surgeonfish, triggerfish, damselfish, and wrasse.

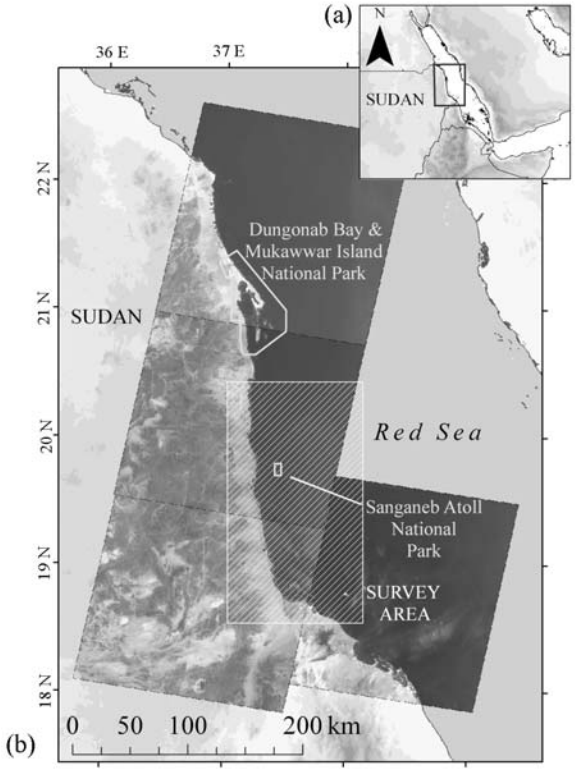

Figure 1: Map showing (a) the location of Sudan (Red Sea) and (b) The Sudanese Red Sea coast and the location of the existing marine protected areas at Dungonab Bay and Mukkawar Island National Park (DBNP) and Sanganeb Atoll Marine National Park (SMNP) and the 2007 survey area (hatched box).

Results

A total of 40 detailed transect sites were surveyed in 2007 and the key findings were as follows:

Benthos

Coral communities in the Sudanese Red Sea support more than 290 species, and transition between those communities characteristic of the northern/central Red Sea and those more typical of the southern Red Sea. Live hard coral cover on the fore reef slopes varied between 11% and 65% (Fig. 2a), and there were notable trends both north to south, and inshore to offshore, while more unusual high cover coral communities were found in sheltered shallow turbid areas. Reefs north of Port Sudan had a significantly lower cover of live stony corals, a higher abundance of both dead standing corals colonised by turfs or coralline algae, and rubble. The extensive mortality of corals on these reefs may of been due to a range of factors (e.g. coral bleaching, Crown-of-Thorns, *Drupella*), but is most likely due to coral bleaching during late 1998, as occurred on the around Dungonab (Kemp et al. 2002) and at similar latitudes on the reefs on the eastern Red Sea (DeVantier et al. 2000a, 2000b). By comparison, southern and offshore reefs supported higher live coral cover and healthy communities of bleaching susceptible species (e.g. *Acropora, Stylophora*), suggesting that these reefs may have avoided the major bleaching events of the past 10 years, most likely due to differences in the local environmental conditions.

Fishes

Pronounced north-south patterns in community structure were apparent, consistent with known patterns in Red Sea biogeography (Fig. 2b). Fish communities however also had very low abundances and diversity of lutjanids (snappers), lethrinids (emperors), serranids (groupers) and scarids (parrotfish) and other groups, which may be a result of overfishing, second-order coral mortality / bleaching effects, or a combination of both these factors (Fig. 2 c). Abundances of exploited species were particularly low in the Suakin archipelago.

Macroinvertebrates

Sea cucumber abundances were very low, suggesting that this group is heavily overfished. This is probably a recent development (within the past 6 to 8 years). Molluscs such as *Trochus* (*Tectis*), *Murex, Lambis* and *Strombus* were absent or present in very low abundance throughout the survey area, whereas *Tridacna* were common or abundant.

Sea urchins (*Diadema* spp. and *Echinometra* matthaei) were almost completely absent in the north and central parts of the survey area, and present only in very low densities in the southern area (Fig. 2 d).

Other Impacts and Threats

Coral diseases and sponge infestations were observed but these were not widespread, although incidents appear to have increased both within Sudan and the wider region in recent years (Klaus pers. obs.). Corallivores such as the Crown-of-Thorns starfish

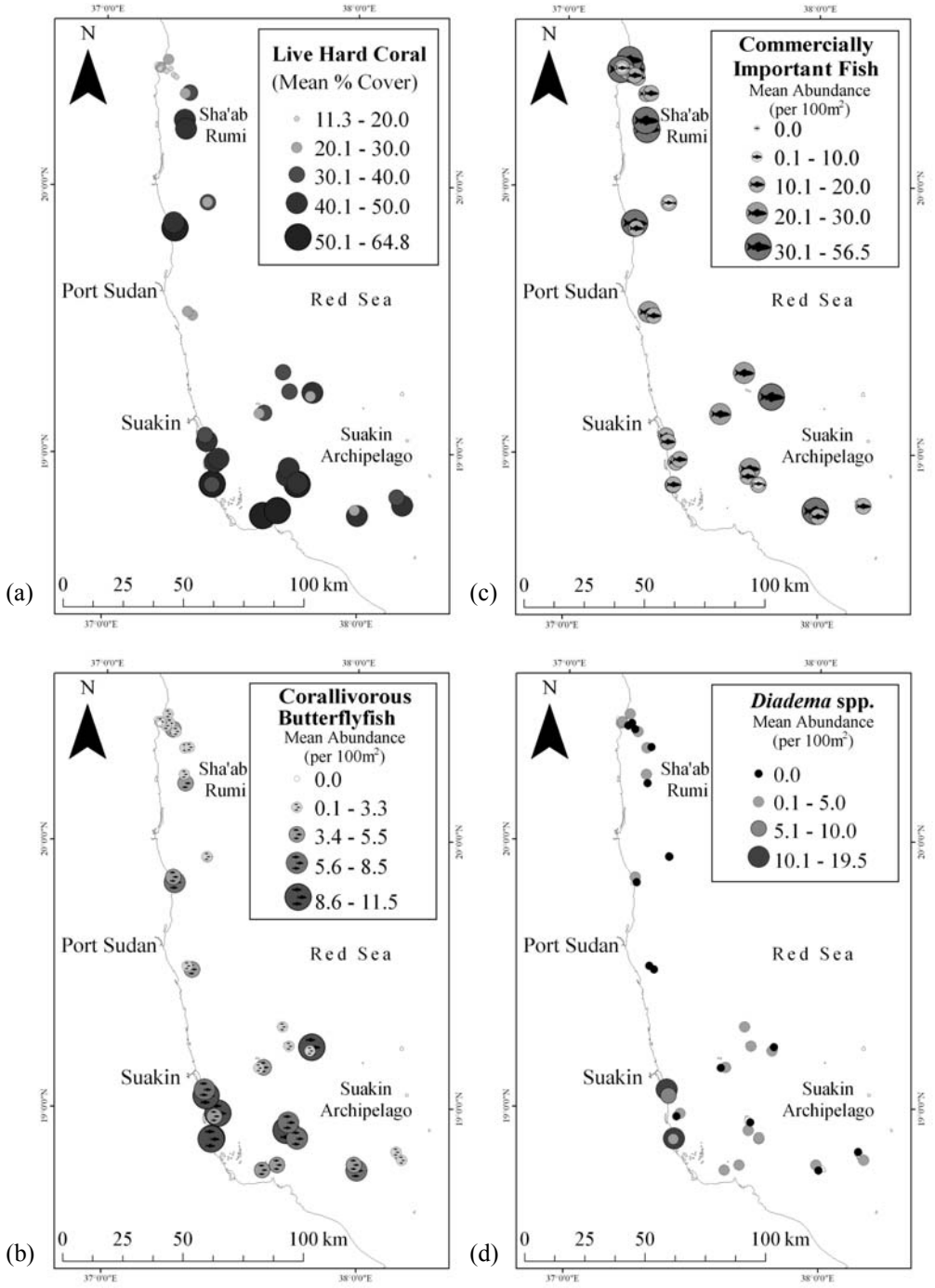

Figure 2: Maps showing some of the key findings from the 2007 survey (a) Mean percent live hard coral cover, (b) Mean abundance of corallivorous butterfly fishes (per 100m^2), (c) Mean abundance of commercially important fishes (per 100m^2) and (d) Mean density of *Diadema* spp. (per 100m^2)

(CoTs), *Acanthaster plancii* and *Drupella* spp. were found at most sites, numbers were generally low, and active outbreaks of CoTs were only found at two sites.

There were low abundances of commercially important fin fish (particularly groupers and large snappers) and invertebrates (sea cucumbers and gastropod molluscs).

Discussion

The composition of the benthic and fish communities found on Sudan's reefs reflects the influences of both the northern and southern Red Sea. Unusual, localized, 'geographically displaced' fish and coral communities may provide opportunities to understand the factors giving rise to biogeographic patterns in the region, and perhaps more widely (Kemp & Klaus, in prep).

The status of the coral communities on both the fringing and offshore coral reefs was highly variable, ranging from poor to good. Reefs to the north of Port Sudan had lower live coral cover and a higher abundance of both rubble and dead standing corals, colonised by turf and coralline algae, and the appearance characteristic of reefs that had been subject widespread coral bleaching event. Although there are few documented observations in the Red Sea during the severe 1997/1998 bleaching event that devastated large parts of the wider Indian Ocean, coral bleaching was observed on the reefs of northern Sudan at Dungonab (Kemp et al. 2002) and on Saudi Arabian reefs at similar latitudes in the eastern Red Sea in August-September 1998 (DeVantier et al. 2000a, 2000b). Corals on fringing and offshore reefs in the Dungonab area were observed to bleach in 1998, and the reefs were reported to be "*so bright white as to be visible from the shore",* while the weather conditions were "*calm and very hot*" (Andrea Bari [*of Dungonab Pearl Oyster Farm*], pers comm February 2002). The first systematic surveys in Sudan following the 1998 event were carried out along a 70 km section of coast in the Dungonab area to the north of Port Sudan in 2002 as part of the baseline surveys for the establishment of Dungonab Bay and Mukawwar Island Marine National Park (Kemp et al. 2002). By comparison, many reefs south of Port Sudan and offshore, showed little evidence of widespread impacts, still supported a high cover of live hard corals, including healthy communities of bleaching susceptible genera, suggesting that these reefs may have avoided exposure to the environmental conditions that caused coral bleaching and mortality on the reefs further north. The patterns in coral cover are reflected in the abundance of corallivorous butterfly fishes and other groups.

Other impacts included CoTs and *Drupella,* although these typically affected smaller patches of reef and their contribution to the spatial variability in the current status of Sudanese reefs was considered to be minor by comparison to the previous bleaching event. Minor outbreaks of CoTs have been recorded in Sudan since the 1970s, and they were present at low abundances throughout the areas surveyed (Kemp et al. 2002, PERSGA/GEF 2004, APF 2006, Cousteau 2007). The most severe outbreak observed during the three surveys undertaken between 2002 and 2007 extended across less than 200m of reef. The widespread coral mortality observed on the northern reefs was concentrated at depths of less than 10m, in contrast to coral mortality caused by CoTs outbreaks in, for example, Sinai, where outbreaks often begin at depths as great as 50m or more and, cause extensive coral mortality to similar depths (Salem 1999). *Drupella* was present at low densities throughout the survey area, and only observed at high densities in very specific habitats in Sudan (shallow areas of high density of *Stylophora* corals) (Kemp et al. 2002, 2003, APF 2006, Cousteau 2007).

Large snappers, groupers (*Epinephelus* spp. and *Plectropomus* spp.) and emperors (lethrinids) are taken by handline fisheries in Sudan. Their absence or very low abundance on most reefs suggests heavy fishing pressure. The low abundance and diversity of parrot fish is less easily explained since they are not targeted in local fisheries. Possible causes may be their dependence on healthy coral reef habitats and lag effects of coral bleaching on fishes (Graham et al. 2007).

The extremely low abundance of many commercially important species of holothurians, and the absence or low abundance of some exploited gastropods suggests that overfishing of these macro-invertebrate groups is widespread.

The complete absence of the common sea urchins (*Echinometra* spp. and *Diadema* spp.) from many reefs is a cause for concern, which reflects the trends recorded by the Reef Check programme (Hodgson and Liebeler 2002), where significant falls in abundance of *Diadema* were recorded on coral reefs of the Indo-Pacific, including the Red Sea, between 1998 and 2001. Historical data on distribution and density of sea urchins in the Red Sea, especially of the important species *Diadema setosum*, is limited, but abundances may be naturally low on some of the offshore deep water reefs. The absence of *D. setosum* from Sanganeb was repeatedly noted by Schuhmacher (1974, 1989) and Kroll, in Schuhmacher *et al. (*1995). The same authors did however note that *Diadema* "is abundant in the northern Red Sea, where it plays a major role in controlling reef development". Reef tops without urchins were, uncharacteristically for the Red Sea, overgrown with small macroalgae, a possible consequence of reduced grazing from both parrot fish and sea urchins. There is no immediately obvious reason for the absence of sea urchins as they were still common in some areas, primarily sheltered, relatively turbid inshore locations such as the large lagoon at Sha'ab Shubuk. These findings require further investigation.

The Future

Until recently, the Sudanese coast has remained

relatively unaffected by the coastal development activities taking place in other countries of the Red Sea. Anthropogenic influences are still mainly limited to a 70 km strip extending from Port Sudan to Suakin, where there are two major ports, oil refineries, a desalination plant, saltworks, power station, a shrimp farm and the new Red Sea Economy Free Trade Zone (although increased development has also recently begun to spread northwards from Port Sudan). The damage to coastal habitats due to construction within this strip is already extensive, and major industrial facilities have been sited with little or no regard to their potential negative environmental impact. Following the peace agreement which brought the war in the south of Sudan to an end, and the recent discovery of oil and gas reserves, rapid coastal development is likely to cause unprecedented economic transformation that will result in a surge in coastal development and associated risks.

Most of the coastal and offshore reef complexes are still relatively undisturbed by human pressures other than fishing. There is a small dive tourism industry, composed of between 8 and 10 boats operating out of Port Sudan and from Egypt. Further rapid development of tourism and other sectors is anticipated as more investors are attracted to Sudan. The increasing development will make the Sudanese coastline more attractive and accessible to the wider foreign and local public. The new coast road from Port Sudan to Egypt is nearing completion, making huge areas of previously relatively undisturbed coast easily accessible, and creating the opportunity for development to the north of Port Sudan but with all the associated risks to coastal habitats.

The focus of future development is likely to continue to be between Port Sudan and Suakin, and the coast between Port Sudan and the Dungonab Bay MPA. The results of this survey suggest that the reefs of Sudan are already suffering from overfishing and coral bleaching mortality, among other impacts. It is therefore of paramount importance that Sudan's designated Marine Protected Areas are given high priority, and that fisheries management plans be implemented. The need for effective planning, and a strategic approach which incorporates considerations of environmental sustainability, is urgent.

Conclusions

Sudan's 750 km long coastline and numerous uninhabited islands and offshore reefs are within the centre of marine diversity in the Red Sea. The country has two MPAs with great potential for UNESCO World Heritage status. Developments in and around Port Sudan are expanding, and the port in this city provides the only maritime access to a country that is otherwise land-locked. The ICZM Programme is uniquely situated to help mitigate future conflicts that may arise over natural resource use, distribution and over-exploitation. This survey is partly responding to the urgent need to provide decision makers with the tools and information necessary to manage the predicted development of the coastal area, whilst protecting its natural heritage.

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The Jamaica Coral Reef Monitoring Network (JCRMN)

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Abstract. The Jamaica Coral Reef Monitoring Network (JCRMN) was launched in June of 2003 by several organizations involved in coral reef monitoring, research and management. The objectives of the JCRMN were to gather information about the coral reefs, stimulate interest in the monitoring of the Jamaican coral reefs, train persons in coral reef monitoring methodologies and to develop a sustainable coral reef monitoring programme. Since its inception monitoring was carried out in the Portland Bight Protected Area, Negril Marine Park, Discovery Bay, Portland, the Port Royal Cays and many other sites around the island. In addition, divers from member ENGOs, hotel resorts and scuba diving clubs, have been trained in the Reef Check Method of coral reef monitoring. The JCRMN has also participated in training and monitoring in Haiti and the Turks and Caicos Islands. Despite the success of the network the development of a sustainable monitoring programme based on external funding and volunteer divers remains the biggest challenge facing the JCRMN. The JCRMN is coordinated through the Caribbean Coastal Data Centre (CCDC) of the Centre for Marine Sciences at the University of the West Indies, Jamaica.

Key words: Coral reef monitoring, Jamaica, JCRMN, GCRMN

Introduction

Jamaica is the third largest island in the Caribbean, after Cuba and Hispaniola, and is comprised of a highland interior with peaks an plateaus running the length of the island surrounded by relatively flat coastal plains (Fig.1). Fringing reefs occur on the narrow 1-2km shelf along most of the north coast and grow sporadically on the much broader shelf (approximately 20km) of the south coast. In addition, reefs and corals are found on neighbouring banks of Pedro Cay and Morant Cay to the south and south west of the island (Burke and Maidens 2004).

Figure 1: Map of Jamaica with insert showing its position in the Caribbean Sea.

The reefs of Jamaica have suffered significant decline since the 1970's. The physical impacts of hurricanes and coral disease combined with the die-off of the grazing sea urchin Diadema in 1983 marked the decline of the Jamaican coral reef. Recovery has been difficult because of the added impacts of human activities which have led to over-fishing, sedimentation caused by soil erosion, nutrient pollution and poorly planned coastal development (Woodley 1992; Hughes 1994). These conditions have been further exacerbated by the ravages of subsequent hurricanes and the effects of coral bleaching.

Rationale for the formation of the JCRMN

The Jamaica Coral Reef Monitoring Network (JCRMN) was launched in 2003 by a number of agencies, institutions and organizations involved in coral reef monitoring, research and/or management (Table 1). The objectives were to develop a vibrant coral reef monitoring network for Jamaica and to provide relevant data and information on the status of Jamaican coral reefs and related ecosystems to inform decision-making at different levels. The formation of the JCRMN was motivated by the existence of the Global Coral Reef Monitoring Network (GCRMN), a network that is involved in coral reef monitoring in 80 countries around the world. The GCRMN functions through a number of independent nodes in various regions of the world. Jamaica is the Coordinator of the Northern Caribbean and Atlantic Node of the GCRMN which also includes The Bahamas, Bermuda, Cayman, Cuba, Dominican Republic, Haiti and the Turks and Caicos Islands.

The Caribbean Coastal Data Centre (CCDC), as the coordinator of the Node, aims to increase the level of collaboration among institutions and countries involved in monitoring within the Node and to assist in improving regional capacity for assessing reef health in the long term and providing appropriate management solutions.

Table 1: Organizations forming the JCRMN

Organizations that have participated in the Jamaica Coral Reef Monitoring Network (JCRMN)

- UWI, Centre for Marine Sciences
- The University Sub-Aqua Club
- National Environment and Planning Agency
- Fisheries Division, Ministry of Agriculture
- The Nature Conservancy
- Negril Coral Reef Preservation Society
- Montego Bay Marine Park
- Friends of the Sea
- Sandals Resorts International
- Beaches Boscobel Resort
- Caribbean Coastal Area Management Foundation
- The Jamaica Sub-Aqua Club
- The Institute of Jamaica
- Mainstreaming Adaptation to Climate Change

Coral Reef Monitoring Methodology

The Reef Check methodology was selected because it is the world's largest volunteer coral reef monitoring programme and provides basic data on the health of coral reefs. Reef Check focuses on the abundance of particular coral reef organisms that best reflect the condition of the ecosystem and that are easily recognizable by non-specialists. The training manuals and field guides are available on their website (www.reefcheck.org) where they also provide technical support (on survey methodology, equipment, data recording, data submission and data analysis) to its registered members. The advantages of the Reef Check method are that it is simple, rapid and inexpensive. This methodology employs low-tech techniques, which facilitates the combination of the manpower of volunteers with the expertise of scientists (Hodgson et al.. 2003). This simple but scientifically robust sampling method has provided data on the condition of reef environments throughout the world and has been adopted as the standard monitoring protocol by marine park managers, national governments, scientific institutions as well as many volunteer teams.

Training and Monitoring

Since the inception of the JCRMN in 2003 approximately 90 persons from various locations around the island, including persons from Haiti and the Turks and Caicos Islands have been trained in the Reef Check Monitoring methodology and have participating in monitoring. Coral reef data has been collected from at least 53 coral reef sites in Jamaica, five sites in Haiti and 18 sites in the Turks and Caicos Islands. Particularly with respect to Jamaica, attempts have been made to monitor the site annually and this has been accomplished at Negril (2001-2006) and Drunkenmans Cay (2003-2007) in Port Royal. The JCRMN was also been able to purchase some equipment and materials to facilitate regular monitoring.

As a result of the activities of the JCRMN the University of the West Indies (UWI) has included the Reef Check methodology in its credited scuba diving course. This association between the UWI and JCRMN provides the opportunity for newly trained divers to participate in a programme that is contributing to the management of the marine environment while allowing them to improve their diving skills.

There is also now an active *egroup* which provides updates and is used to coordinate the volunteers. Regular meeting of the JCRMN are held to evaluate the progress of the network, plan for future activities and explore funding opportunities. The data collected is archived at the CCDC and also sent to Reef Check International. The data is used to prepare reports for the CCDC and other members of the network (e.g. Linton 2003a and 2003b; Linton et al. 2003; Jones 2006, NEPA 2008) and also used in contributions to the *Status of the Coral Reefs of the World* reports (Jones et al. 2004, Creary et al. in press) and the "*Status of Caribbean Reefs after Bleaching and Hurricanes in 2005*" (Jones et al. 2008) publication produced by the GCRMN,. The achievements of the JCRMN have also been the subject of newsletters, workshops and conference presentations.

Funding

Funding has been provided by ICRAN Project of the UNEP-CEP and the British High Commission (in Jamaica) to conduct the training and monitoring exercises in a number of sites in Jamaica and in Haiti. For the training exercises that took place in Negril and Boscobel accommodation were provided by Sandals International, Beaches Resorts and other hotels in the area. DEFRA (Department for Environment, Food and Rural Affairs, UK) provided the funding to monitor the coral reefs within the Portland Bight Protected Area and has also made funds available to carry out additional training and monitoring as well as the hosting of a stakeholders workshop (Creary and Jones 2007) to

report on the work of the JCRMN. In kind support is provided by the participating individual and agencies of the JCRMN.

Selected Results of the JCRMN Monitoring Programme

Jamaica

The Portland Bright Protected Area (PBPA), the largest protected area in Jamaica (1,876 km^2) is located on the south coast of Jamaica and is currently managed by the Caribbean Coastal Area Management Foundation (CCAM) a members of the JCRMN. The marine area (approximately 70% of the PBPA) has widespread coral reefs associated with the 16 cays and several shoals (Linton et al.. 2003). Extensive surveys were conducted by the JCRMN in conjunction with CCAM during 2004 and 2005 at nine sites associated with the cays and shoals. The results from these assessments shows variable hard coral cover ranging form 0% to 34% with a mean of 20% (Fig. 2). Between 8 and 13 coral species were identified and the most common species were those of *Porities spp* and *Montastrea spp*. At the site with no hard coral present the substrate was dominated (48%) by algae. Sea urchin densities were generally low but parrotfish were abundant at most sites (Jones 2006).

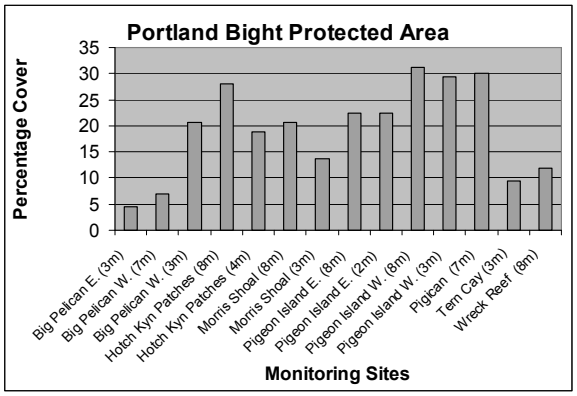

Figure 2: Hard coral cover at sites within the Portland Bight Protect Area, Jamaica.

Four sites located within the boundaries of the Negril Marine Park have been monitored annually since 2001. The sites (Ireland Pen, Bloody Bay, El Punto Negrilo and Little Bay) are all exposed and experience varying levels of fishing pressure and anthropogenic impact (Linton 2003a). Fig. 3 provides the most recent results (2006) of the assessments carried out at shallow and deep locations at the sites in Negril. Coral cover was low ranging from 6.3 to 18.1% with an overall mean of 11.5%. At all sites with the exception of Bloody Bay the deeper sites had higher coral cover.

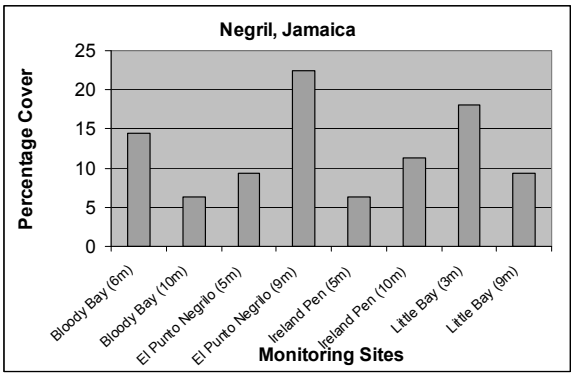

Figure 3: Hard coral cover at four sites in the Negril Marine Park, Jamaica

Haiti

A team led by the JCRMN visited Haiti during the period September 15-20, 2003 to assist the Foundation pour la Protection de la Biodiversite Marine (FoProBIM), a Non-Governmental environmental organization with the assessment of reef sites at Les Arcadins. A two-member team from Haiti was trained during this period. Surveys were conducted at 5 sites at two depths. The sites were: Trou Forban, Ti' Teal, Ilé Fa, Ilé Sud and Bo wout Montrouis.

Visual surveys of the sites revealed generally healthy reefs. They appear to be in reasonably good condition, with the exception of some sites that had relatively high percentage of nutrient indicating algae, suggesting some anthropogenic impacts. Preliminary data analyses indicated a range of coral cover from a high of approximately 50.0% to a low of 7.0% (Fig. 4); cover by Nutrient Indicating Algae (NIA) was also relatively low. Fish populations were low at all sites ranging from 0.5 to 5.0/ 100 m^2 (Linton 2003b).

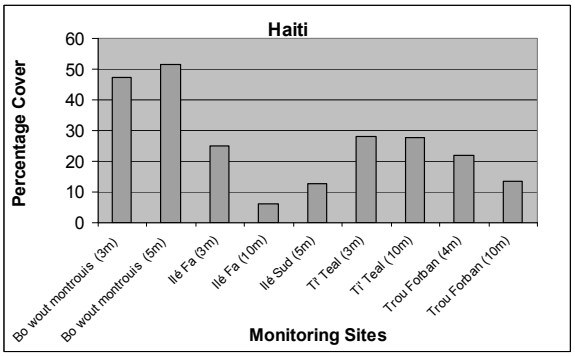

Figure 4: Hard coral cover at five sites assessed in Les Arcadins, Haiti.

Turks and Caicos Islands

During August 3-19, 2007, the Department of Environment and Coastal Resources of the Turks and Caicos Islands, along with members of the

JCRMN completed a wide scale survey and assessment of 18 sites around the major islands to establish the current state of the coral reef resources. These results showed that live coral cover on hard bottom averaged about 16% with a range of 6.3–38.8% (Fig. 5).

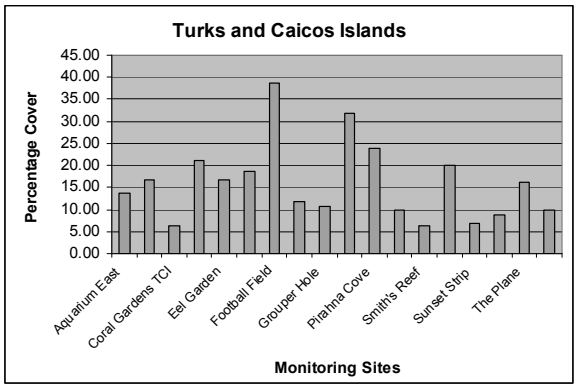

Figure 5: Hard Coral Cover for 18 sites in the Turks and Caicos Islands.

Challenges faced by the JCRMN

Despite the successes there have been challenges faced by the JCRMN in the implementation of a comprehensive monitoring network in Jamaica.

The logistics of organizing volunteers can often be problematic. As most of the work has to be done on the weekends monitoring is limited to two or three days at the most. If monitoring is to be carried out during the week volunteers need to request time off from work. Even those who work in the environmental field have to find time in their work schedule to participate in the JCRMN activities, separate and apart from their regular work schedule. Volunteers also use their own equipment and the maintenance and replacement of these is not factored into the monitoring programme.

The varying diving and species/substrate identification skills of the divers also have to be taken into consideration. Often times the more skilled divers carry out the majority of the monitoring and are sometimes called on to finish up the data collection initially undertaken by the less-skilled divers. It has also been observed that for those divers who are not scientists, their interest in doing "work" dives wanes after a number of monitoring exercises because the monitoring does requires focus and concentration to complete the task within a specified time (before the divers becomes exhausted or air gets too low).

Funding is needed to ensure the continuity of the monitoring programme. Although the volunteers are not paid, funds are required to purchase monitoring equipment and supplies and to cover the cost of transportation, accommodation and meals. In-kind contributions are helpful but tend not to be sustainable. In addition to coordinating the activities of the JCRMN data processing and report preparation is presently carried out by the CCDC, which is presently understaffed.

The Future of the JCRMN

The JCRMN has established a schedule of monitoring primarily focused on the annual assessment of existing sites giving priority to the marine protected areas. Provision has also been made to add new sites for which no information exists. Reef Check training for recently trained divers (those trained through USAC and UWI) who are interested in becoming JCRMN volunteers and also for members of the other interested agencies will continue.

In the longer term efforts are being made to obtain funds to support the permanent position of JCRMN Coordinator whose responsibilities would be to develop the interest of the group and to coordinate activities geared towards establishing and implementing the framework for coral reef monitoring in Jamaica. Additional funding would also be required to continue training and monitoring and to support the establishment of additional monitoring networks in the other islands of the Northern Caribbean and Atlantic Node of the GCRMN.

Conclusion

The Jamaica Coral Reef Monitoring Network through a cadre of trained volunteer divers has managed to reduce the costs associated with coral reef monitoring. Although there are challenges regarding manpower and funding the members of the network are committed to facilitating its long-term sustainability.

Acknowledgment

The JCRMN could not have achieved the success that it has with out the contribution of the member organizations and volunteer divers. **Jamaica -** Noylis Amair, Brooke Anderson, Kerrith Armstrong, Richard Baddal, Devon Brown, Damany Calder, Channeil Cadogan, Ansurd Carey, Michael Clarke, Wayne Clarke, Norman Clayton, John Cleland, Tameka Clough, Donald Comer, Monique Curtis, Lauren DeFino, Krishna Desai, Edward Downer, Rachel D'Silva, Raymond Earle, Lyle Edwards, Peter Edwards, Tracey Edwards, Anthony England, Nadia-deen Ferguson, Iona Flett, Sheena Francis, Peter Gayle, Linvel Getten, Elfred Greenfield, Mark Gold, Marlon Goldson, Sean Green, Dave Guiness, Kapleton Hall, Tamia Harker, Brandon Hay, Avril Hazlewood, Ainsley Henry, Marlon Hibbert, Nicholas Jack, Kimberly John, Loureene Jones, Ivanna Kenny, Matthew Lee, Jahsen Levy, Dulcie Linton, Michael Loftin, Kristoffer Lue, Suresh Martin, Elizabeth McGhie, Michelle McNaught, Judith Mendes, Malden Miller, Bryan Murray, Tadaomi Nakai, Minke Newman, Jenny Pearce, Shanti Persaud, Andre Pitter, Clayton Powell, Norman Quinn, Marsha Robinson, Dana Roeber, Andrew Ross, Chalene Roye, Daniel Scarlett,

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Session number 18

Coral Bleaching Mortality and Resilience at Stetson Bank, a High Latitude Coral Community in the Gulf of Mexico

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Abstract. Stetson Bank, located near 28.2° N, 94.3° W in the northwestern Gulf of Mexico, approximately 110 kilometers offshore of Galveston, Texas, is an uplifted claystone feature associated with an underlying salt dome. Due to the influence of the warm, clear waters of the Gulf Stream Loop Current, Stetson Bank supports a well-developed benthic community comprised of tropical marine sponges, coral and other invertebrates. Living coral cover, predominately *Millepora alcicornis*, can comprise up to 30% of the reef surface. A long-term monitoring program was initiated in 1993 consisting of 66 permanently marked stations on the reef surface that are photographed annually and analyzed for changes in community structure and benthic cover. In the summer of 2005, significant mortality of coral was documented in association with a major bleaching event in the Gulf of Mexico. In addition, the area was subject to significant coastal runoff resulting from two major hurricanes (Katrina and Rita). The synergistic effect of both coastal runoff and elevated temperature may have contributed to mortality in 2005. Here we present an analysis of monitoring data from 2004-2008 and evidence of possible resiliency of the coral-sponge community of Stetson Bank.

Key Words: coral bleaching, resiliency, Gulf of Mexico, Millepora, Stetson Bank

Introduction

Stetson Bank is one of a number of topographic features associated with underlying salt domes located in the northwestern Gulf of Mexico (Fig. 1; Rezak et al. 1985). Rising from surrounding water depths of over 55 meters, Stetson Bank crests to within 15 to 25 meters of the sea surface. The main feature of Stetson Bank is made up of uplifted claystone and siltstone pinnacle formations that harbor a well developed coral-sponge community. Stetson was first described in the 1950's (Carsey 1950; Stetson 1953; Neumann 1958) due to increasing interest of the oil and gas industry in offshore oil exploration along the continental shelf.

The clay/siltstone pinnacle structures of Stetson Bank support a unique coral and sponge community. Surrounded by a clay-mud bottom, the pinnacles rise to within 15 meters of the water's surface, providing structure above the turbid depths into the relatively clear, warm surface waters. The predominant benthic species on Stetson Bank is the hydrozoan *Millepora alcicornis* (fire coral) accounting for up to 30% of the reef surface in the pinnacle area. In addition, eleven other species of hermatypic corals have been documented, including *Diploria strigosa*, *Stephanocoenia intersepta*, *Madracis mirabilis*, *Madracis decactis*, and *Agaricia fragilis*. Sponges, primarily *Chondrilla nucula*, *Ircinia strobilina* and *Agelas clathrodes*, comprise up to another 30% of the benthic cover. A running total of 180 species of fish, including several species of sharks and rays (Pattengill 1998), 644 species of invertebrates, including mollusks and echinoderms, and 2 species of turtle (*Caretta caretta* and *Eretmochelys imbricata*) also inhabit this coral-sponge community (see http://www.flowergarden.noaa.gov/ document_library /science/recdivingspecieslist.pdf).

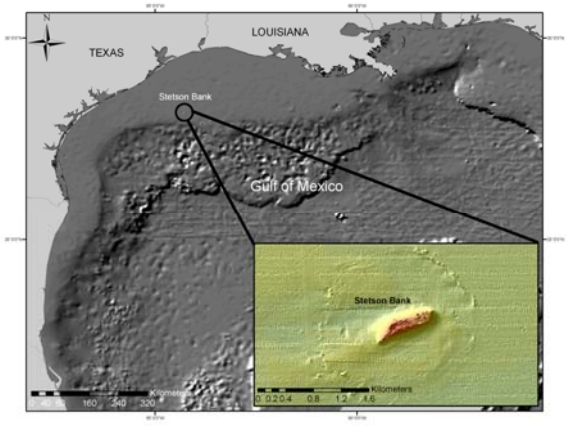

Figure 1: Location of Stetson Bank in the northwestern Gulf of Mexico. Inset shows bathymetry of Stetson Bank and surrounding deeper ring of siltstone pinnacles. Bathymetry provided by USGS/Gardner.

In 1996, Stetson Bank was incorporated into the Flower Garden Banks National Marine Sanctuary under revisions to the Marine Protection, Research

and Sanctuaries Act of 1972 (now the Marine Sanctuaries Act). Since 1993, a long-term monitoring program has been in place, utilizing permanent photographic stations, fish counts and random transects, as well as temperature and salinity probes. Initially, 66 long-term photographic stations were established, of which 44 station locations are currently known. Long-term monitoring cruises occur every summer, in June or July, weather permitting.

In 2005, a Caribbean wide bleaching event (for more information, see http://coralreefwatch.noaa.gov /caribbean2005/) severely impacted Stetson Bank, coupled with two hurricanes (Katrina and Rita) which caused mechanical damage as well as an increase in coastal runoff. A dark plume from the Mississippi River and coastal Louisiana encircled the banks for a few weeks after Hurricane Rita passed (Fig. 2). Here we present an investigation into the amount of change and degree of resiliency of Stetson Bank after this widespread bleaching event and repeated impacts from two hurricanes, through an analysis of five years of monitoring data, from June 2004 to June 2008.

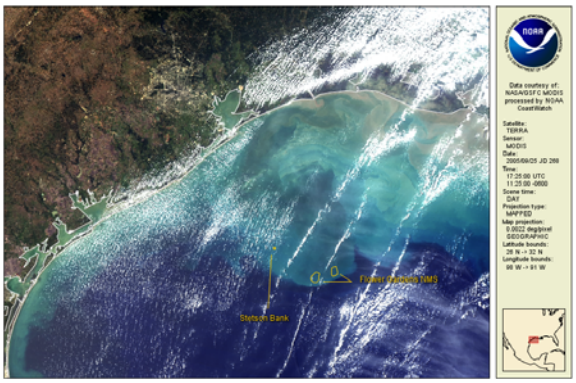

Figure 2: Satellite imagery showing the plume of coastal runoff elicited by Hurricanes Katrina and Rita. The plume moved over 100 miles south into the Gulf of Mexico, directly over Stetson and the Flower Garden Banks. This image is dated 25 September 2005, one day after Hurricane Rita's landfall. Data is courtesy of NASA/FSFC MODIS, processed by NOAA Coast Watch.

Material and Methods

Long-term monitoring cruises were conducted during the weeks of June 14 – 16, 2004, June 13 – 15, 2005, June 19 – 21, 2006, July 22 – 25, 2007, and June 16 – 18, 2008. From 2004 through 2007, a total of 51 different stations were photographed with a Nikonos V camera, with a 15mm lens, and two Ikelite 200 underwater strobes, mounted on a 1 meter T-frame with a bubble-leveler and compass. 100 ASA Fujichrome Sensia II film was used. The Nikonos was focused to 2.6 feet, f 8, on auto-exposure. The area of coverage was 1.6 m^2. Slides were commercially developed and scanned at 1200 pixels per inch (Nikon LS1000). In 2008, a Nikon Coolpix P5000 digital camera, housed in an Ikelite underwater housing, with two Ikelite DS125 sub-strobes and an Ikelite wide angle lens adapter, replaced the Nikonos V film camera. The digital camera was mounted on a modified T-frame (1.25 m in length), which provided a slightly larger coverage area than the Nikonos V. After digital images were downloaded, image distortion was removed using Photoshop CS2 software (V9.0.2, Adobe Systems Inc.), and then the image was cropped to maintain 1.6 m^2 coverage, the coverage obtained by the Nikonos V. Benthic cover was determined from a random overlay of 30 points using Coral Point Count with Excel extensions software (CPCe V3.5; National Coral Reef Institute (NCRI), Florida; Kohler and Gill 2006).

Sea surface temperature data were obtained from a National Data Buoy Center (NDBC) 3-meter discus buoy (Station 42019, 27.91˚ N, 95.36˚ W), located 60 nautical miles south of Freeport, Texas and 58 nautical miles west-southwest of Stetson Bank. The NDBC buoy reads the water temperature at 0.6 m below the water surface and is positioned over a water depth of 82.3 m. Bottom temperatures were obtained from HOBO temperature loggers (HOBO Water Temp Pro [H20-001]) and data sondes (YSI 6600 Series) placed on the crest of Stetson Bank at 23.5 m depth.

Statistical analysis

Percent cover values for major species categories (i.e., Coral, Sponge, Algae, Coralline Algae, and Other) could not be transformed to conform to a normal distribution. Therefore, percent cover data for each major species category were analyzed using non-parametric Friedman's ANOVA. Photographic stations which were not consistently photographed for all five years were not included, which left 39 out of 51 stations for the analysis. All tests were performed using Statistica software (6.0; StatSoft, Inc.).

Results

Changes in percent benthic cover

Percent coral cover changed significantly between 2004 and 2008 (Friedman's ANOVA; $\chi_{37,4} = 85.63$, $P < 0.001$), decreasing dramatically after 2005 (Table 1). Specifically, *Millepora alcicornis* decreased by 80% from 2005 levels and has not recovered as of 2008 (Fig. 3). Other hermatypic coral species have shown minor fluctuations in percent cover from 2004-2008, but were generally stable (3.5% in 2005; 4.2% in 2008).

Algal cover also changed significantly over the five years ($\chi_{37,4} = 82.93$, $P < 0.001$). In general, algal cover increased since 2005, from 29% to a peak of 63% in 2007, but decreased to near 2004 levels at 49% in 2008. Specifically, percent cover of *Dictyota*

fell from a high of 26% in 2006 to 3% in 2008, while turf-algae matrix increased to a high of 36% in 2007, and then decreased to 5% in 2008. Coralline algae (Corallinaceae) increased from 2% in 2004 to almost 9% in 2008 ($\chi_{37,4} = 29.58$, $P < 0.001$).

Sponge cover also changed significantly over time ($\chi_{37,4} = 19.72$, $P < 0.001$), decreasing from 27% in 2005 to a low of 18% in 2007. However, in 2008, overall sponge cover increased to 23%. Sponge species showed differing trends in cover. *Chondrilla nucula* decreased from 5% in 2005 to 0.2% cover in 2008. In contrast, *Ircinia strobilina* remained between 8-10% cover, and *Agelas clathrodes* between 1-2% cover between 2004 and 2008.

'Other' percent cover, which includes bare substrate, substrate rubble and mobile organisms, also changed significantly over time ($\chi_{37,4} = 15.74$, $P = 0.003$), decreasing from 10% to 6% in 2006, and then increasing to 12% in 2008.

	June 2004		June 2005		June 2006		July 2007		June 2008	
	% cover	s.e.m.	% cover	s.e.m.	% cover	s.e.m.	% cover	s.e.m.	% cover	s.e.m.
Millepora alcicornis	22.90	2.69	24.82	2.68	5.79	1.12	5.52	1.04	3.43	0.82
other corals	3.66	0.18	4.53	0.21	3.35	0.19	3.75	0.21	4.14	0.20
Chondrilla nucula	5.28	0.40	3.65	0.31	0.63	0.08	0.98	0.10	0.26	0.07
other sponges	15.39	0.09	23.26	0.13	18.81	0.12	16.81	0.11	22.89	0.13
Dictyota spp.	25.74	3.46	19.62	2.84	26.04	3.37	22.60	2.65	2.20	0.56
Turf-algae matrix	7.64	1.52	4.17	1.44	29.20	2.88	36.30	2.64	4.58	1.01
other algae	11.77	0.51	6.34	0.30	4.97	0.32	3.74	0.29	40.67	1.36
Coralline algae	1.79	0.28	2.86	0.33	4.25	0.41	4.09	0.39	8.71	0.73
Bare substrate	4.30	0.65	7.46	1.09	5.33	1.01	5.25	0.89	10.65	1.31
Substrate rubble	0.73	0.73	3.21	1.17	0.54	0.33	0.53	0.37	1.67	0.84
Other	0.65	0.09	0.09	0.02	1.08	0.08	0.44	0.05	0.79	0.07

Table 1: Percent benthic cover, averaged over all photostations, (± standard error, s.e.m.) from 2004 through 2008. The bleaching event occurred during the summer of 2005. Category 'other' in the table represents urchins, fish and other organisms which appeared under the random point generated by CPCe software.

Changes in temperature and salinity regime

Sea surface temperatures reached a peak in 2005, with water temperatures spiking to 30°C or greater on 67 days of the year (Table 2). Winter temperatures were also considerably milder than other years, with only 27 days falling below 20°C. In comparison, 2006 reached similar high, as well as low, sea surface temperatures, though for not as many days as in 2005.

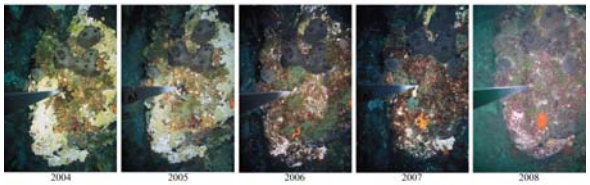

Figure 3: An example from one long-term photostation from 2004 to 2008. An obvious shift from *Millepora* to algae and sponge-dominated substrate is apparent in this series of photographs. In 2004 and 2005, *Millepora* (yellow coloration) is shown in relative abundance on the pinnacle, but is overgrown by algae in 2006 and 2007.

Sea surface and HOBO temperatures showed seasonal fluctuations over the mid-shelf area and Stetson Bank, respectively (Fig. 4 A, B). HOBO probes reported lower temperatures on the crest of Stetson than nearby, mid-shelf sea surface temperatures obtained from the NDBC surface buoy (e.g., August 7, 2005; Fig. 4A: 31.29°C SST; Fig. 4B: 29.26°C HOBO). Further, there were several sudden drops in temperature evidenced by the HOBO dataset, which were not reflected in the SST dataset (e.g., July 15, 2005: 29.8°C SST, 24.6°C HOBO). These discrepancies could be explained by the occurrence of cold water thermoclines over Stetson Bank during the summer months.

Year	Number of days SST reached 30 C	Number of days SST fell below 20 C	Mean temperature (± s.e.)	HIGH	LOW
2004	34	63	24.980 ± 0.044	31.6	15.2
2005	67	27	25.157 ± 0.040	33.1	18.8
2006	40	19	25.036 ± 0.037	33.1	18.8
2007	27	59	24.881 ± 0.039	31.8	18
2008*	7	48	22.977 ± 0.049	31.9	18.3

Table 2: Temperature records from Stetson Bank from 2004-2008. Asterisk denotes temperature data was not calculated from a full year; data is shown up to June 30, 2008.

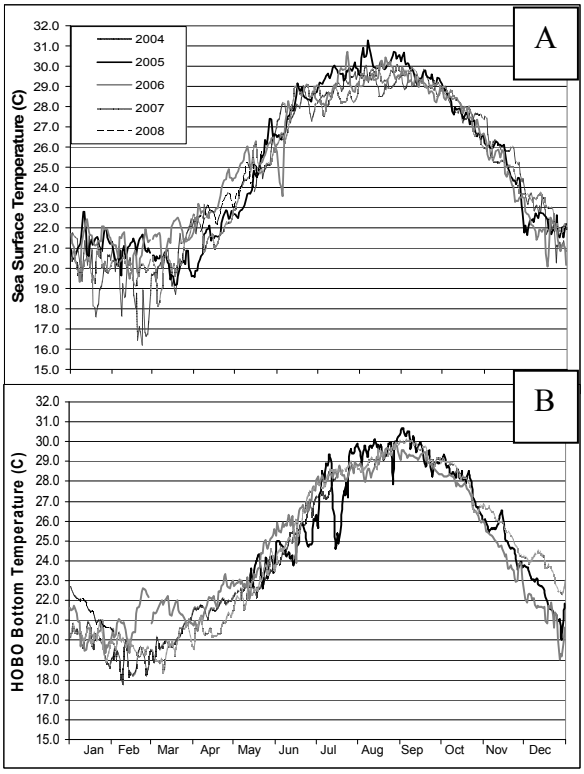

Figure 4: Average daily temperatures from 2004 – 2008. (A) Sea surface temperatures from NDBC Buoy 42019. (B) Bottom temperatures from the surface of Stetson Bank (23.5 m depth) from HOBO temperature probes.

Salinity was not measured as consistently as temperature due to instrument difficulties. However, a particular salinity regime emerged from the datasonde measurements. There were repeated freshwater influxes to Stetson Bank between 2004 and 2008

(data not shown). However, freshwater influxes in 2005 seemed to be of greater duration and extent than in the other years of this study. To contrast the extent of the lower salinity regime in 2005, in 2006 the average yearly salinity was 35.3 ppt, compared to 32.9 ppt in 2005. Throughout the end of July and August of 2005, daily average salinity measurements were as low as 27 ppt, and with the passage of Hurricane Rita, salinity again decreased from an average in September of 35 ppt to an average daily low of 32 ppt on October 2 (see Fig. 5).

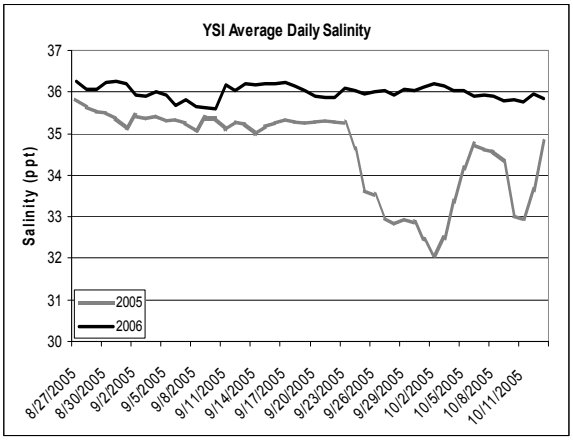

Figure 5: Average daily salinity measured between August and October, 2005 and 2006. Salinity was measured with a datasonde/YSI on the surface of Stetson Bank (23.5 m depth). Hurricane Katrina passed through the Gulf of Mexico on August 28, 2005; while Hurricane Rita passed through the Gulf on September 23, 2005.

Discussion

Though previous studies have suggested that the benthic community of Stetson Bank is relatively stable (Bernhardt 2000), this study is the first to report a dramatic change in percent cover of Stetson Bank's benthic community. This analysis allows for investigation into questions regarding the mechanisms of recovery or resiliency of this high-latitude coral-sponge community.

Several trends in percent cover are apparent from the analysis of the five years surrounding the bleaching event of 2005. Prior to the Caribbean wide bleaching event in 2005, Stetson Bank supported 27% coral cover. After 2005, overall coral cover fell to 7%, with *Millepora* suffering the largest decrease and not recovering as of the 2008 monitoring effort. The other hermatypic coral species of Stetson Bank (e.g., *Diploria strigosa, Stephanocoenia intersepta, Madracis decactis, Madracis mirabilis*, and *Siderastrea radians*) appeared stable, remaining at around 4% of the benthic cover throughout 2004 to 2008. This demonstrates the significant differential response of *Millepora* to bleaching compared with other species of hermatypic coral. The composition of sponge species varied between 18-27% throughout the five years. However, there was a dramatic decrease in *Chondrilla nucula* percent cover after 2005. Bleaching in sponges is not well documented (see Vicente 1990; Fromont and Garson 1999) and though we can not say from this dataset that the mortality of *C. nucula* was due to bleaching, *C. nucula* virtually disappeared between the 2005 and 2006 monitoring cruises. Further, the disappearance of *C. nucula* at Stetson Bank is an opposite response from the increase in *C. nucula* cover reported from the reefs of Belize after the 1998 coral bleaching episode (Aronson et al. 2006). Along with the varied success of the sponge species, there was an initial increase and subsequent decrease in algal cover. Concomitant with this decrease in filamentous and macro-algae, coralline algae and turf-algae matrix has steadily increased since 2005.

Although the benthic community has not recovered to pre-2005 levels, trends observed in this study suggest possible signs of resiliency of the reef ecosystem of Stetson Bank. An increase in coralline algae, relatively stable hermatypic coral and sponge cover and decreasing macroalgal cover (after an initial spike) have been cited as possible signs of reef resiliency elsewhere (see Birrell et al. 2005; Smith et al. 2008). However, understanding the details of the disappearance of both *Millepora alcicornis* and *Chondrilla nucula* and the interaction of the overall biological community with trends in temperature and salinity regimes is equally important in determining the overall resiliency of Stetson's *Millepora*-dominated community.

The temperature data from Stetson Bank demonstrate differences in accuracy between the sea surface (SST) and bottom (HOBO) temperature measurements. There was a dramatic decrease in summertime bottom temperature in July 2005, coupled with bottom and sea surface temperatures which repeatedly reached over 30°C. Temperatures of 30°C and above are considered to signal the probable onset of bleaching for tropical corals, depending on the latitude of the reef system and seasonal maximum SST (Manzello et al. 2007). Further, Degree Heating Week (DHW) data, which is a proxy for thermal stress and represents the number of weeks during which the average weekly sea surface temperature is greater than the expected average monthly maximum temperature, also showed 2005 to be remarkable in the temperature regime over Stetson Bank (for a more thorough explanation of DHW, see http://coralreefwatch.noaa.gov/satellite/methodology/methodology.html#dhw). Specifically, compared to 2004, with 24 weeks at 0.55 DHWs, there was a dramatic increase to 32 weeks at a maximum of 6.05 DHWs in 2005. In 2006, there were only 6 weeks at

0.5 DHW; 2007 increased to 25 weeks at 0.8 DHW. As of September, there were no DHW in 2008 (data courtesy of NOAA Coral Reef Watch).

Coupled with the high, prolonged temperatures of 2005, coastal runoff from the passage of Hurricanes Katrina and Rita also reached Stetson Bank. Though Stetson Bank is subject to repeated influxes of freshwater, none of the documented influx events between 2004 and 2008 rivaled the events which occurred in 2005. The lower salinity regime instigated by two hurricanes passing through the northern Gulf of Mexico lasted several weeks, from August to October (see Fig. 5). Though water quality was not specifically tested at the FGBNMS, this plume of freshwater originating from the Gulf Coast and Mississippi River and driven southwestward by the winds of Hurricane Rita and spinoff eddies from the Loop Current, might have carried pollutants, chemicals, and nutrients to an already stressed reef community (see Rabalais et al. 1996; for review of effects of coastal discharge on corals, see Fabricius 2005).

The lack of quick recovery of *Millepora* following the bleaching event might be attributed to the dependence of the population on larval recruitment, rather than colony regrowth. Due to the severity of the 2005 bleaching event, most *Millepora* colonies exhibited total mortality, so that regeneration from remaining live tissue was not possible. An initial image assessment of a subset of randomly selected photostations exhibited only one or two new colonies between 2007 and 2008. *Millepora* spawning has not been reported from the FGBNMS, and little is known about its reproductive ecology (see Soong and Cho 1998). Depending on local *Millepora* behavior and larval duration, the resiliency of the *Millepora* population of Stetson Bank might depend on larval recruits from other coral communities in the Gulf of Mexico. However, there is limited research on the extent of population connectivity among the reefs and banks of the northwestern Gulf of Mexico (but see Lugo-Fernandez 1998; Lugo-Fernandez et al. 2001).

High seawater temperatures and major hurricane impacts (with associated freshwater influxes) in 2005, followed by an increase in recruitment-inhibiting macroalgae species (e.g., see Kuffner et al. 2006), and a dependence on larval recruitment, might explain why the reef community of Stetson Bank has not yet recovered to pre-bleaching benthic species assemblage patterns. However, as of 2008, the reef ecosystem of Stetson Bank may be exhibiting signs of resiliency, as evidenced by a subsequent decrease of macroalgae and an increase in coralline algae, the degree of which will be established through continued monitoring efforts.

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Spatial and temporal recovery patterns of coral reefs within the Gulf of Oman (United Arab Emirates) following the 2007 cyclone disturbance

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Abstract. In June 2007 Cyclone Gonu, the most powerful storm in the region in 60 years, impacted reefs in the United Arab Emirates along the Gulf of Oman coastline. The extent of destruction of the reef corals varied considerably depending on site, taxa and colony morphology. This study describes the short-term recovery of corals following the cyclone disturbance. Monitoring stations have been installed near the cities of Dibba and Fujairah. Each station consists of two primary components; (i) transect markers that create a three-ray pattern for repetitive photographic surveys, and (ii) a settlement plate rack with horizontal and vertical limestone tiles. Analyses of the transect photographs provide spatial and temporal comparisons such as coral area cover, colony size frequency distributions and colony growth rates. Examination of the settlement plates indicates coral larvae recruitment into the area, which may also indicate future shifts in community structure. Site data such as hourly seawater temperatures measured approximately 0.5m above the reef, sea urchin and crown-of-thorn starfish densities, and rugosity (topographic complexity) are evaluated in relation to the spatial and temporal patterns. While this study focuses on the short-term recovery of these reefs, future studies may continue to track their long-term progression at the permanent monitoring stations.

Key words: Cyclone Gonu, hurricane disturbance, recovery, Gulf of Oman.

Introduction

Coral communities in the eastern United Arab Emirates (northwestern Gulf of Oman) exist in shore-parallel patches and around rock islands. Coral cover in this area has typically been 30-40% at depths of 4-12m. The overall condition of corals in the Gulf of Oman had been relatively stable prior to June 2007. Upwelling mitigates elevated SST anomalies that lead to bleaching elsewhere and storm damage has been uncommon. On occasion, coral communities have experienced localized temporal variability due to crown-of-thorns starfish outbreaks and periodic recruitment episodes (Rezai et al. 2004).

In June 2007, Cyclone Gonu hit the Arabian Peninsula as the strongest storm since recordkeeping began in 1945. Following such a disturbance, coral community structures are reshaped based on the recovery of the surviving colonies and the recruitment of new individuals into the community (Hughes 1989; Smith and Hughes, 1999; Riegl 2001; Loch et al. 2003; Lirman 2003; Golbuu et al. 2007; Guzman and Cortes 2007). Positive recovery outcomes include the continued growth of undamaged colonies, fragment reattachment, and remnant tissue regrowth on partial-mortality colonies. Negative recovery outcomes include subsequent mortality due to disease resulting from compromised colony health, transportation of fragments to unsuitable locations, and the collapse of reef framework resulting from bioerosion. Recruitment of new individuals may be the result of sexual reproduction of local surviving colonies or the immigration of larvae that have dispersed over long distances. Sexual reproduction of local surviving colonies may be negatively impacted by sublethal stresses that require increased energy expenditure for maintenance, repair and growth. Fecundity may be decreased and reproductive cycles may be interrupted, delayed or skipped in such colonies (Harrison and Wallace 1998).

The objective of this study was to compare the short-term recovery patterns between and among the coral communities offshore Fujairah and Dibba, UAE. Data collected from permanent monitoring stations shall be used to describe the community structures of the surviving colonies, as well as the recruitment patterns of new individuals into the population.

Methods

Monitoring stations were installed in areas representing various levels of disturbance along

coastal Dibba and Fujairah, United Arab Emirates. The monitoring stations consist of three replicate 10.0m x 1.5m belt transects in a three-ray pattern (Fig. 1). A settlement plate rack serves as the center point of the station. Three transect markers are cemented into the nearby reef, approximately 12m from the center marker and at 120° angles from each other. Temporary lines are strung from the center marker to each of the end markers to create the three rays of the star pattern. These transect markers ensure the repeatability of photographs and benthic measurements (i.e. rugosity, slope, depth, sea urchin abundance) by marking the start and end points of the surveys.

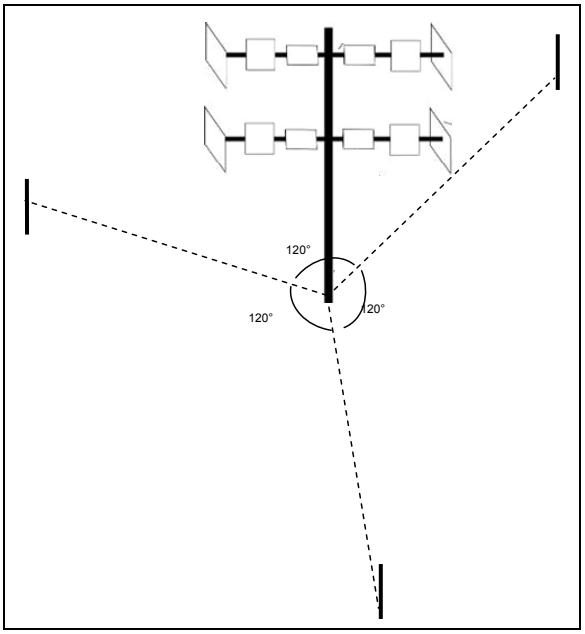

Figure 1: Three-ray monitoring station design. A settlement plate rack serves as the center point. Transect markers are permanently cemented into the reef to create the three rays of the star pattern.

Limestone or terra cotta tiles (10cm x 10cm x 1 cm thick) were attached to the settlement plate rack. The tiles were positioned (i) horizontally, (ii) vertically and parallel to the rack, and (iii) vertically and perpendicular to the rack. Tiles in positions (i) and (ii) were in pairs to create unshaded upper and shaded lower surfaces as well as cryptic space in the gap between the tiles. Tiles will be replaced annually and examined for coral spat.

Digital images were be taken along each of the transect rays using housed Olympus C-750/C-770 cameras (4 mega pixel) and 16 mm wide-angle conversion lenses set 50 cm above the reef inside rigid photo-framers. These photographs were analyzed for hard coral area cover. Image mosaics were created and individual colony surface areas were be traced and color coded by species to create a visual representation of each transect ray for spatial and temporal comparisons. The hard corals in each photograph were identified to species and surface areas (planar view) determined using Coral Point Count with Excel extensions (CPCe) (Kohler and Gill 2006). The data to be derived from CPCe included percent live coral cover, coral density as individuals per m^2, number of species present, average colony size, size frequency distribution, and diversity indices.

Results

The cyclone disturbance had varying effects on the coral communities depending on site, taxa and colony morphology. The initial assessment of the four monitoring stations in August 2007, two months following Cyclone Gonu, indicated that resilient taxa (i.e. faviids and poritids) were minimally damaged, while the susceptible taxa (i.e. acroporids and pocilloporids) were moderately to heavily damaged. The least damaged sites, Dibba South (DIBB2) and Mirbah North (MIRB1), included areas with massive colonies of *Platygyra* and *Favia* spp. that survived relatively unscathed, showing little or no signs of scouring or partial mortality. Dibba Rock (DIBB1) was a moderately damaged site that included areas of dense monospecific stands of *Pocillopora damicornis* from which individual colonies were dislodged or broken. Mirbah South (MIRB2), an area that had been dominated by tabular *Acropora clathrata,* was the most severely damaged site with large sections being completely razed.

Benthic data collected from the monitoring stations in August 2007 are shown in Table 1. Rugosity ratios (topographical complexity) measured 1.1-1.2, indicating the near flattening of reefs (1.0 indicates a horizontal surface). Live coral cover ranged between 24-45%, primarily due to the survival of massive colonies and the presence of detached fragments with live tissue. Coral densities varied between sites (5.2 – 33.3 colonies per m^2), with higher densities being the result of extensive breakage and fragmentation of branching and bushy colonies. The average colony surface area ranged between 98-452 cm^2, with the lower averages occurring at sites with extensive fragmentation.

Between 7-14 species were inventoried along the transect rays at each site (Table 2). This was higher than anticipated, based on previous site visits, because less common cryptic taxa were now obvious due to the removal of the branching and bushy coral overstories. Four species of massive scleractinians (*Favites pentagona, Platygyra daedalea, Porites lutea, Siderastrea savignyana*) were present at all four monitoring stations.

Table 1: Benthic data collected at monitoring stations in August 2007. Sites are presented from north to south, with DIBB1 being the northernmost site and MIRB2 being the southernmost site.

	DIBB1	DIBB2	MIRB1	MIRB2
Rugosity Ratio	1.1	1.2	1.1	1.1
Live coral cover (%)	45	24	30	33
Coral density (colonies per m^2)	18.7	5.2	9.5	33.3
Average colony surface area (cm^2)	239	452	314	98
Sea urchin density (individuals per m^2)	0.6	2.2	0.6	0.0

Table 2: Taxa present at monitoring stations in August 2007. Species inventories are conducted along the transect rays at each monitoring station.

	DIBB1	DIBB2	MIRB1	MIRB2
Acropora clathrata	**X**		**X**	**X**
Coscinaria columna		**X**		X
Cyphastrea microphthalma		**X**		
Favia pallida		**X**	**X**	**X**
Favia speciosa		**X**		X
Favites pentagona	**X**	**X**	**X**	**X**
Platygyra daedelea	**X**	**X**	**X**	**X**
Pocillopora damicornis	**X**			X
Porites harrisoni	**X**	**X**		X
Porites lutea	**X**	**X**	**X**	**X**
Porites solida			**X**	**X**
Porites nodifera		**X**		X
Psammacora spp.				X
Psuedosiderastrea tamyami				X
Siderastrea savignyana	**X**	**X**	**X**	**X**

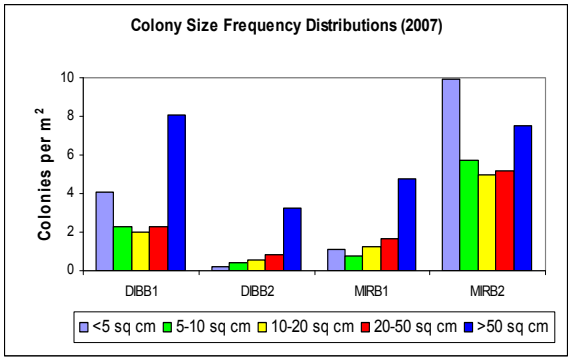

Figure 2: Colony size frequency distributions in August 2007. Large massive colonies (>50 cm^2 diameter) survived at all stations. Large fractions of small individuals (<5 cm^2 diameter) were present where extensive fragmentation occurred during Cyclone Gonu.

Size frequency distributions demonstrated the survival of the large massive colonies (>50 cm^2 diameter) at all monitoring stations (Figure 2). Large fractions of small individuals (<5 cm^2 diameter) were present at DIBB1 and MIRB2 where extensive fragmentation of *Pocillopora* and *Acropora*, respectively, occurred during Cyclone Gonu.

Sea urchin densities ranged from 0.0-2.2 individuals per m^2 along the transect rays (Table 1). Multi-year sampling shall indicate if this is a normal population or if the abundance was reduced by the cyclone. Sea urchin densities are important as these herbivores maintain the standing algal crop that may otherwise outcompete coral recruits for available substrate.

No crown-of-thorn starfish (COTS) were observed along the transect rays or within the general vicinities of the monitoring stations. COTS have been shown to preferentially feed on branching taxa (McClanahan et al 2005). Therefore, the presence of COTS may influence the recovery of taxa that are also susceptible to mechanical damage from cyclones (i.e. acroporids and pocilloporids). If COTS are observed in subsequent surveys, COTS densities shall be calculated for each site and compared with recovery patterns for branching taxa.

Discussion

The results presented herein are from the first of several surveys to be conducted in order to compare the short-term recovery patterns between and among the coral communities offshore Fujairah and Dibba, UAE. Rugosity and coral cover may increase over time if fast-growing species (i.e. acroporids and pocilloporids) recover quickly. Coral densities may fluctuate; increasing if recruits settle onto newly available substrate or decreasing if fragments are transported elsewhere by water movement. Taxa inventories may change with the settling of additional species from distant larval sources or if localized extinctions occur from post-storm circumstances such as algal competition or disease outbreaks. Size frequency distributions are expected to vary primarily based on the outcomes of the *Acropora* and *Pocillopora* fragments since the massive colonies, which grow at rates of ~1 cm per year, are unlikely to move between size classes for the duration of this study. The skewness in size frequency distributions can indicate trends in recruitment, survival and growth of coral communities. Communities skewed to the left (i.e. mostly larger colonies) may indicate strong survival of massive colonies with poor post-disturbance recruitment. Communities skewed to the right (i.e. mostly smaller colonies) may indicate extensive fragmentation or heavy recruitment.

While this study focuses on the short-term recovery of these reefs, future studies will track long-term progression at the permanent monitoring stations.

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Bathymetric Distribution of the Benthic Marine Flora in Chemuyil, Mexico

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Abstract. 34 species of benthic marine flora between 0.5 and 35 ms of depth were determined, species with the greatest dominance was: *Enteromorpha flexuosa, Halimeda incrassata, Coralline articulate, Lobophora variegata* and *Galaxaura oblongata, E. flexuosa* was the most common. The sampling methodology was based on 117 phototransects to different depths. The index of diversity and density increased with the depth. The most frequent species with relative abundance bigger than 10% were *E. flexuosa* and *H. incrassata*. In this work the importance of some ecological attributes in the bathymetric distribution for this group was analyzed. The depth with greater diversity was registered at 3 m. The analysis of assemblaje gives a high associations among stations, their diversity and specific richness, which evidence the importance of such factors as: depth, light availability, substratum, wave effect, sedimentation and environmental conditions, among others.

Key words: algae, macroflora, distribution, biodiversity, Yucatan.

Introduction

The vegetation is one of the main components of coralline reefs with multiple functions, one of the most significant is the stabilization of substratum, because it is the nutritional resource of a great number of herbivorous and a fixing device of vital elements such as nitrogen, which recycles and makes it available for other groups. Some genus, such as *Halimeda* and *Penicillus* deposit aragonite (as $CaCO_3$) forming sand and sediment, participate actively in the reef formation or in change processes.

In reef ecosystems, marine flora is represented by macro algae which are a group with high diversity. It is important to evaluate reef communities with emphasis in the structure of their populations, information necessary to formulate accurate hypotheses of the relation between the biotic and abiotic factors of these environments (Liddell and Ohlhorst 1991). Also, the present study analyzes the composition and space distribution of the macroalgae in the coralline reef of Chemuyil, Quintana Roo at different depths.

Material and Methods

Study Area. The reef of Chemuyil is a barrier located 115 km from Cancun in the coast of the State of Quintana Roo, Mexico. It is part of the northwest region of the Caribbean zoogeographic province (Fig. 1).

Sampling. The sampling took place by means of an annual cycle from 2006 to 2007, with 117 photographic transects of 20 m of length (Liddell and Ohlhorst 1991, Torruco 1995) and stations each meter, work was performed in 14 depths of the reef between 0.5 and 35 m. At the same time, a selective collection was made for its identification in the laboratory (Taylor 1960, Litter et al. 1989) and it was used as base of reference for the photo-interpretation.

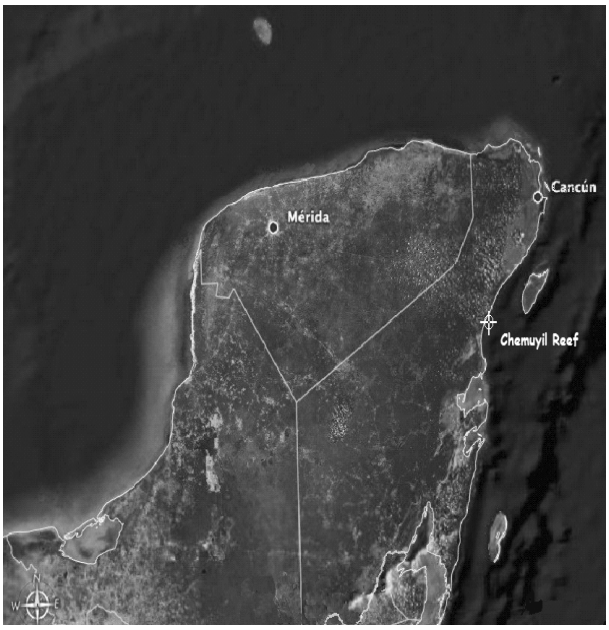

Figure1. Map showing the Chemuyil Reef, Quintana Roo, Mexico.

Analysis. The Dominance analysis was determined following Orlóci hierarchic criterion (Orlóci, 1978), the diversity was evaluated by means of the index of Shannon-Wienner (Magurran, 1988) and eveness with the equation of Pielou (1984). In the cluster analysis the index of similarity of Bray-Curtis was used with Lance and Williams criterion of flexible union with a β=0.25 (Orlóci, 1978). The discrimination of the species by depth was evaluated with the divisive analysis of arrangement using the content of information of order 2 (Ezcurra and Equihua, 1984).

Results

A total of 34 species of benthic marine flora between 0.5 and 35 m were determined. The ten more dominant species were: *Enteromorpha flexuosa* (16.54), *Halimeda incrassata* (15.55), *Lobophora variegata* (8.58), *Galaxaura oblongata* (8.45), *Dictyota divaricata* (6.31), *Amphiroa rigida* (4.86), *Halimeda opuntia* (4.32), *Caulerpa racemosa* (3.03), *Centroceras clavulatum* (2.77) and *Dictyota dichotoma* (2.45).

The highest specific richness was registered at 6 m of depth (S=12); followed by 3 m, 5 m and 35 m (S= 11), the lowest one appeared at 32 m (S= 4). Also, the most diverse depth corresponded to 6 m with H= 2.98 bits/ind and the lowest to 3 m with H= 1.23 bits/ind. In the eveness the pattern was the same, the highest at 6 m (E= 0.83) and the lowest at 3 m (E= 0.35, Fig. 2).

The similarity analysis registered three assemblajes at higher levels than 80 %. The first one at a 0.9 level links to 6 and 18 m depths, stations characterized by their high specific richness (S= 12 and 10 respectively) and diversity (H= 2.98 and 2.6 bits/ind). The second at a level of 0.85 associated mainly by the proximity of depth to the 13 and 14 m, with a variable specific richness (S= 6 and 10 respectively) and an intermediate diversity (H= 1.74 y 1.86 bits/ind).

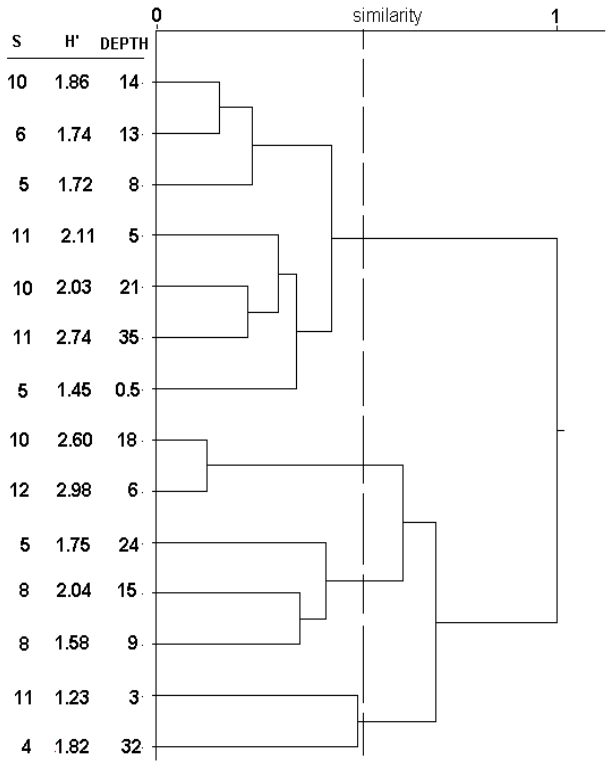

Figure 2. Cluster analysis of depth similarity, richness of species and diversity in Chemuyil Reef, Quintana Roo, Mexico.

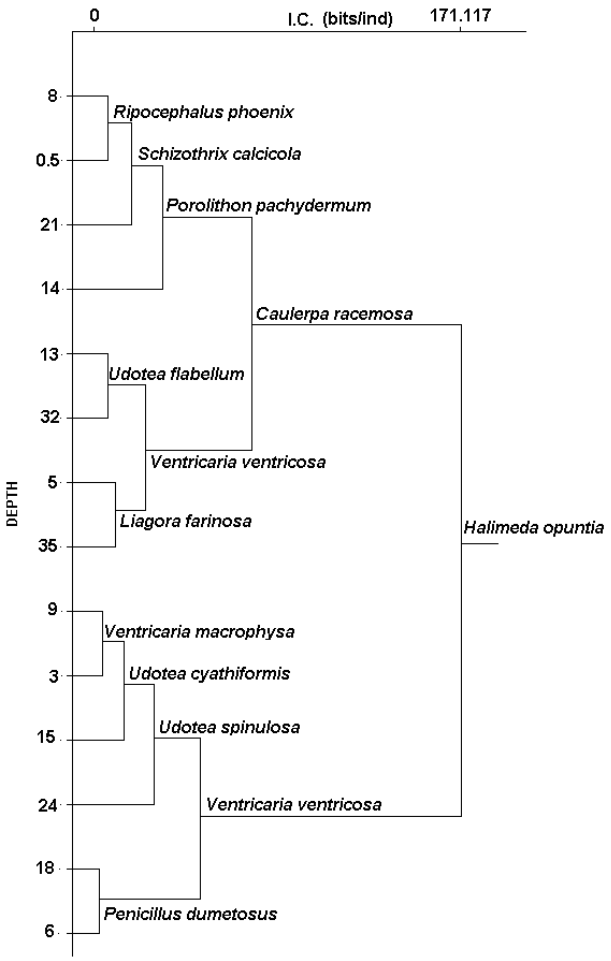

Figure 3. Cluster analysis of macroflora by discriminate the species in Chemuyil Reef.

The third at 0.80 level associated to high depths: 21 and 35 m, was characterized to register high specific richness (S= 10 and 11 respectively) and diversity (H= 2.03 and 2.74 bits/ind). These associations evidence high diversity and specific richness in the reef at high depths (Fig. 2).

The analyses of assemblage by depth to discriminate the species are shown in figure 3. The seven indicating species of the discrimination between the levels 0.07 to 0.87 are in sequential order: *Halimeda opuntia, Caulerpa racemosa, Ventricaria ventricosa*, *Porolithon pachydermum*, *Udotea spinulosa*, *U. cyathiformis* and *Schizothrix calcicola*. *Liagora farinosa* was the indicating species at 0.9 level and at greater levels than 0.93 the discriminating species were: *Ripocephalus phoenix*, *Udotea flabellum*, *Ventricaria macrophysa* and *Penicillus dumetosus*, this last species associated to the depths of 6 and 18 m, characterized by its high diversity and specific richness.

Discussion

The marine benthic macroflora of the barrier of Chemuyil was less diverse in comparison with other reefs areas of the region (Table 1). This result can be because the comparative one was made with protected natural zones, which provides a greater diversity and abundance. Other causes can be the sampling method used or a greater competitive pressure, due to the ample coralline development of the area.

Table 1. Comparison of the richness in the touristic corridor Cancun - Tulum. C= Chlorophyta, P= Phaeophyta, R= Rodophyta and S= Sorce.

Areas	C	P	R	S
Costa Occidental de I. Mujeres, P. Cancún y P. Nizuc	86	44	124	INE 1998a
Sian Ka'an	66	19	86	INE 1996
Sian Ka'an	15	46	35	Keeney 1999
Cozumel	75	55	166	INE 1998b
Chemuyil	17	7	10	This Work

Great part of the coast of Quintana Roo, is a combination between sandy and rocky beaches, specially the shallow reef areas, which originates an ample variety of habitats. The analysis registered an ample development of coral scleractinian in the platform, between their furrows and crests (63 % of the total area), reason why a competitive pressure with the macroflora is considered. The most conspicuous species were the calcareous forms of red algae, which reached 5 %, the total coverage of the group in the reef was of 24 %. Gutierrez-Carbonel et al. (1995) reports in the Reserve of the Biosphere of Sian Ka' an greater coverage (50 to 65%). The registered assemblies provide greater stability, the obtained pattern of high richness and diversity appeared to high depths and decreases towards intermediate depths. The indicating species of the state of health of the macroflora in Chemuyil were: *Enteromorpha flexuosa* and *Halimeda opuntia*.

Other authors (Cramer 2008, Gilner and Van Woesik 2008, Venera-Ponton et al. 2008), mention that the reefs of the Caribbean are being transformed into algae reefs; however the reef of Chemuyil registered a greater coralline development between the 30 and 40 ms of depth, although it is certain that the greater richness and diversity of macroflora appeared to high depths, the percentage of coverage was minor to the one of the coralline coverage, reason why this asseveration must be referred to shallow zones. Consequently, the richness of species and the obtained patterns of diversity probably exhibit low registries, they increase as depth increases, due to the high rate of sedimentation registered in the shallow zones, which originates a reduction in their biodiversity.

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Continued degradation of Tobago's coral reefs linked to the prevalence of coral disease following the 2005 mass coral bleaching event.

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Abstract. In the summer of 2005, the Caribbean region experienced a widespread coral bleaching event. In order to determine the extent and effect of this event on the reefs of Tobago, the Buccoo Reef Trust (BRT) and Coral Cay Conservation (CCC) conducted a targeted coral reef survey programme around the island between October and November 2005 as the first part of a two-phase campaign. In Phase I the reefs were assessed to establish their current status. In Phase II, a long-term monitoring programme was installed to record subsequent degradation or recovery of the reefs from the bleaching event. Results from Phase 1 found overall mean bleaching of hard corals to be 66%. Although most sites had greater than 85% bleaching, some local reefs on the northeast of Tobago had less then 20% bleaching, perhaps indicating localised tolerance. Bleaching was also found to be highly variable, both between and within species. Notably, *Acropora palmata* stands all appeared to be unbleached. No significant coral diseases were observed during Phase 1 surveys. Results from Phase 2 indicate a high prevalence of coral disease at many sites throughout the area. Yellow Band Disease was commonly seen on *Montastrea* species. There were also high levels of White Plague Disease and *Aspergillosis*. Initial findings suggest that disease were generally prevalent on reefs adjacent to areas of high coastal development and/or agricultural land use and prevalence was inversely related to depth.

Keywords: Coral, disease, bleaching, reef status, Tobago

Introduction

The coral reefs of Tobago, like many of those throughout the Caribbean region, are severely threatened (Gardner et al. 2003) and the overriding cause is thought to be human activity (Mora 2008). Threats to reef health include overfishing, habitat degradation, land-based pollution stresses and climate change induced events. The prevalence and types of coral disease in the Caribbean have been well documented (Antonius 1981; Goreau et al. 1998; Green and Bruckner 2000; Smith et al. 1996; Sutherland et al. 2004). Tobago has been no exception with serious disease outbreaks in the past e.g. Laydoo (1983).

The large-scale bleaching event of 2005 is an example of a major climate change induced event that significantly affected Tobago's coral reefs (see Wilkinson and Souter 2008). In order to determine the extent and effect of this event on the reefs of Tobago, the Buccoo Reef Trust (BRT) and Coral Cay Conservation (CCC) conducted a targeted coral reef survey programme at 22 sites around the island between October and November 2005.

A two-phase campaign was launched. In Phase I the reefs were assessed to establish their current status. In Phase II, a long-term monitoring programme was installed in order to monitor the reefs to record subsequent degradation or recovery from the event.

Results from Phase 1 found overall mean bleaching of hard corals to be 66% (O'Farrell and Day 2005). Bleaching levels in Tobago were found to be consistent with regional results, with most Tobagonian sites exhibiting greater than 85% bleaching. However, some local reefs on the northeast of Tobago had less then 20% bleaching, perhaps indicating localised tolerance. Bleaching was also found to be highly variable, both between and within species. Notably, *Acropora palmata* stands all appeared to be unbleached. No significant coral diseases were observed during Phase 1 surveys although a few diseased coral colonies were observed in north-west Tobago in 2003 including White Band, Yellow Band (Blotch) and Black Band Disease (Lapointe 2003).

Following the successful partnership in 2005 CCC and the BRT established a programme of

study in collaboration with the Tobago House of Assembly and with the support of the United Nations Development Programme Global Environment Fund (UNDP-GEF). Part of this study, the Tobago Coastal Ecosystem Mapping Project (TCEMP), began in March 2007 in north-west Tobago (Fig. 1) with the three main objectives to:

1. Provide baseline data on the status of Tobago's coral reef, mangrove and seagrass ecosystems;
2. Increase awareness about Tobago's marine resources through educational programmes;
3. Build in-country capacity through scholarship programmes.

The data collected during the TCEMP will be used by the Integrated Watershed and Coastal Area Management (IWCAM) Caribbean programme to provide relevant information to the Government of Trinidad and Tobago in order to enable effective coastal zone management.

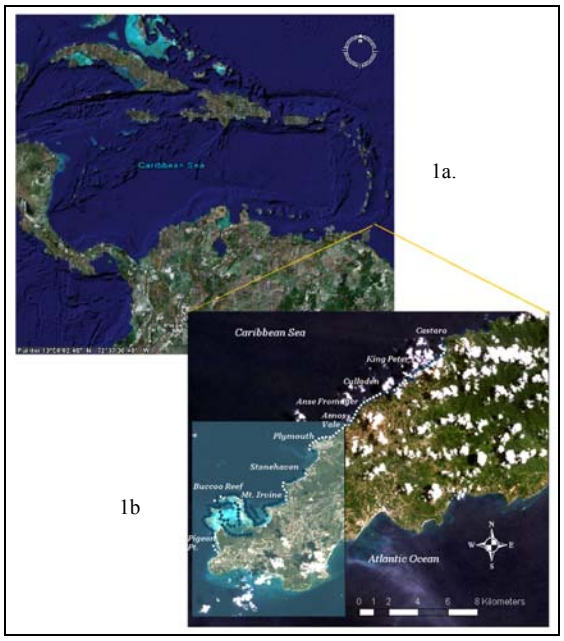

Figure 1. Map of the Caribbean (1a) and western half of Tobago (1b) indicating the area of study

Methodology

Coral Disease Studies

Anecdotal observations suggested that coral disease prevalence had become a more serious contributor to coral reef degradation in Tobago following the 2005 bleaching event. To document this phenomenon, monitoring of the prevalence and severity of common and easily identifiable coral diseases was initiated in 2007. Here we present some initial trends for diseases recorded during CCC baseline surveys. Monitoring stations of tagged coral colonies were also set up in order to collect quantitative data on the rate of coral disease infestation. Full details of the survey methods used are provided in the report by van Bochove et al. (2008).

Results

All data presented was collected on CCC baseline surveys between May 2007 and June 2008 along the north-western coast of Tobago between the Buccoo Reef complex and Castara (Fig. 1). Four coral diseases, Yellow Blotch Disease (YBD), White Plague (WPL), Dark Spot Syndrome (DSS) and Aspergillosus (ASP), were recorded most often on surveys conducted at 7, 12 and 16 metres depth (Table 1). Other coral diseases that were recorded occasionally were Black Band Disease (BBD), White Band Disease (WBD), White Pox (WPD) and Patchy Necrosis but data for these diseases are not presented here.

Table 1. Coral species affected by four main diseases in north-west Tobago.

Disease: Species	Yellow Blotch	White Plague	Dark Spot	Asper-gillosus
Colpophyllia natans		M, D		
Dichocoenia stokesii			M	
Diploria strigosa	S, D	S, M		
Gorgonia ventalina				S, M, D
Millepora sp.	D			
Montastraea spp.	S, M, D	M, D	M	
M. faveolata	S, M, D	S, M, D		
M. franksi	S, M, D	S, M	D	
M. cavernosa	S, M, D	S, M	D	
Porites asteroides		S, M, D		
Siderastrea radians			S	
Siderastrea siderea		M, D	S, M, D	
Solenastrea hyades	D			

Where: S = Shallow (7 m), M = Medium (12 m), D = Deep (16 m).

Ten species of hard corals, one hydrozoan and one gorgonian were affected by the four main coral diseases (Table 1). A number of genera were infected by more than one disease at a range of depths (e.g. *Montastraea* and *Sideastrea*). Others were only affected by one disease type such as *Porites asteroides* (WPD) and *Gorgonia ventalina* (ASP).

Coral disease prevalence for the four main diseases is presented in Figure 2 for three depth bands (7, 12 and 16 metres) as a mean percentage value for all the coral types affected. The proportion of coral colonies affected by YBD,

WPL and DSS appear to be inversely related to depth, with the highest levels recorded at the shallowest depth band surveyed (7 m).

This decrease with increasing depth is most obvious for DSS where disease prevalence decreases from 67% to 23%. The highest levels of disease prevalence were recorded for common sea fans with *Aspergillosus* (72%) Infestation levels were all greater than 60% of coral colonies at the shallow depth band (7 m).

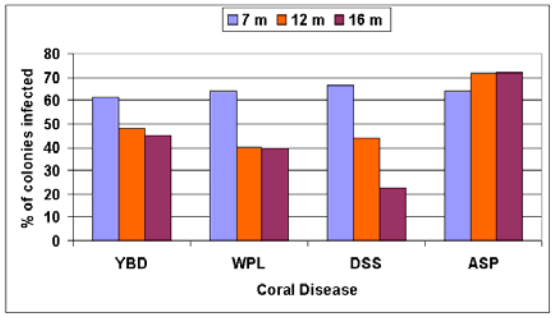

Figure 2: Coral disease prevalence in all coral types at three depth bands in north-west Tobago

Looking at two hard coral genera in detail, coral disease data for *Montastraea* and *Siderastrea* indicates that each genus is mainly affected by two types of disease (Figs. 3 & 4).

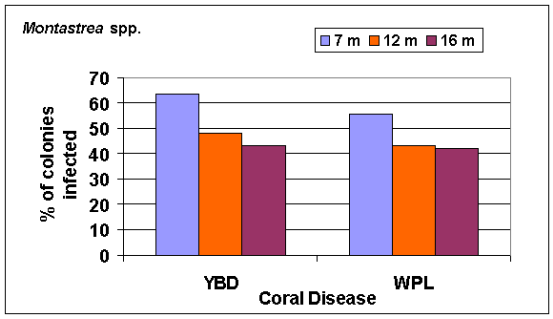

Figure 3: Coral disease prevalence in *Montastraea* spp. at three depth bands in north-west Tobago

Coral disease prevalence was generally higher in more shallow waters. More than 60% of *Montastraea* colonies were infected with YBD in shallow waters (7 m) with prevalence decreasing to 43% (16 m). Similarly prevalence of DSS in *Siderastrea* was high (67%) at 7 m. depth but dropped off markedly to 24% at 16 m (Figure 4)

Statistical comparison of disease prevalence for *Montastraea* and *Siderastrea* colonies over the three depth bands and for three diseases (Table 2) revealed significantly higher percentages of colonies infected in shallower waters for YBD in *Montastraea* and DSS in *Siderastrea* (One-way ANOVA and Two-tailed T-Test on arcsine transformed data).

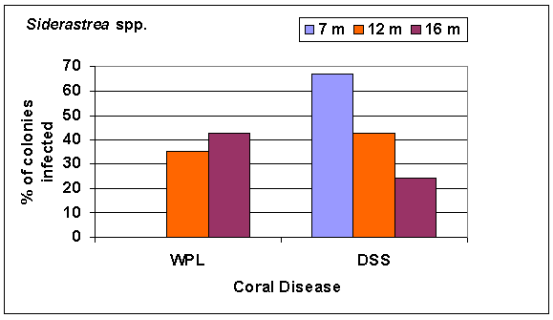

Figure 4: Coral disease prevalence in *Siderastrea* spp. at three depth bands in northwest Tobago

Table 2. Statistical comparison of coral disease prevalence for two coral genera and three diseases over three depth bands

2a – One-way ANOVA

Genus	Disease	P value
Montastraea	YBD	< 0.001
Montastraea	WPL	n.s.
Siderastrea	DSS	< 0.001

2b – Two-tailed T-Test

		Depth Comparison		
Genus	Disease	7 vs. 12	7 vs. 16	12 vs. 16
Montastraea	YBD	++	+	n.s.
Siderastrea	DSS	++	+++	++

Where: n.s. = not significant; + = $p<0.05$; ++ = $p<0.01$; +++ = $p<0.001$

Summary and Conclusion

Coral disease is certainly a serious issue for the fringing reefs of north-western Tobago and is likely to cause a further reduction in live hard coral cover for a region that is already suffering from high coral mortality, particularly after the 2005 bleaching event. Indeed the increase in coral disease prevalence since the bleaching event may be directly linked to the thermal stress experienced during 2005 in that climate-mediated, physiological stresses may compromise host resistance and increase frequency of opportunistic diseases (Harvell et al. 1999).

This study has indicated that coral disease prevalence for YBD and DSS appears to be related to depth with the percentage of coral colonies infected decreasing with increasing depth for *Montastraea* and *Siderastrea* respectively. At the shallow depth band (7 m) at least 60% of coral

colonies were infected by one or more disease for the coral species recorded. The positive relationship between depth and disease prevalence for certain combinations of genera and disease types may well be linked variation in water quality and land-based pollution (Bruno et al. 2003). Alternatively deeper corals are likely to have experienced less bleaching in 2005 and may therefore be less susceptible to infection.

Four main coral diseases (YBD, WPL, DSS and ASP) were recorded along Tobago's northern coastline. However, insufficient data was available at the time of writing to accurately compare coral disease prevalence on a spatial scale between reef sectors along the coast. There is also a need to conduct a temporal analysis of the data to determine whether disease prevalence is changing over time, especially since the last mass coral bleaching event. Further analyses will be undertaken as the TCEMP progresses and the dataset becomes more robust. An interesting future comparison will be to look at the level of coral disease prevalence between the north-west and north-east coasts of Tobago as the two areas differ in terms of population density and coastal development. The north-east coast was also less severely bleached than the north-west in 2005, which may also influence coral disease prevalence.

Acknowledgements

Coral Cay Conservation and the Buccoo Reef Trust would like to acknowledge the kind support and partnership of the Tobago House of Assembly in the TCEMP as well as all partners and supporters of the project both in the UK and the Caribbean, but particularly UNDP and the GEF Small Grants Program. CCC would also like to thank all the dedicated field staff and volunteers on the project site who enable the TCEMP to run effectively.

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Small-scale variations in the effects of coral bleaching in Rodrigues

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Abstract. Rodrigues was one of the few reef locations in the Western Indian Ocean to escape the mass coral bleaching event of 1997 – 1998, however shallow reef sites have suffered from bleaching events in 2002, 2005 and 2007. In all 3 years, there were small-scale spatial variations in the severity of bleaching and associated mortality. Temperature data indicate small-scale variations in sea temperature around the lagoon and current data suggest that bleaching tended to occur in areas with the lowest current speeds. Recovery of sites from bleaching also showed small-scale spatial variations. Inshore sites, displayed limited recovery to the beaching events and sites are dominated by turf algae (>60 %) with low live coral cover (<15 %), whereas live coral cover has increased at a site further offshore. This variation in recovery is likely to be due to the proximity to land and increased human impacts. This suggests that if coral bleaching events continue to occur on a regular basis then these inshore reefs could become overgrown by algae and their structural complexity eroded. Rodrigues is a small, isolated island with a limited coral larval supply and such a scenario would leave the island vulnerable to coastal flooding and storm damage.

Key words: coral bleaching, mortality, recovery, sea surface temperature, Indian Ocean

Introduction

Rodrigues is a semi-autonomous island forming part of the Republic of Mauritius, located 560 km north east of the main island in the western Indian Ocean. The island is surrounded by 90 km of fringing coral reef, which creates a shallow lagoon of 240 km^2. One hundred and sixty species of coral have been recorded in Rodrigues (Fenner et al. 2004) and the fore reefs have high live coral cover (30 – 50 %) at 6 – 12 m depth (Shoals Rodrigues unpublished data).

The coral reefs of Mauritius and Rodrigues were some of the few reef areas in the Indian Ocean to escape the mass coral bleaching event of 1997 - 1998 (Turner et al. 2000). However, unusually warm and calm conditions occurred during February 2002 resulting in coral bleaching, particularly at sites in the north and west of Rodrigues (Hardman et al. 2004). Surveys showed that although the bleaching was not widespread, it was severe where it did occur, resulting in mortality of up to 75 % of corals at some sites. Further coral bleaching occurred in 2005. Bleaching was again most severe in the north and west of the island, where up to 50 % of coral bleached, although mortality was not so severe as in 2002 (Hardman et al. in press).

This study investigates whether further coral bleaching occurred during 2007, following another period of warm, calm weather in January of that year. It also examines spatial variations in the recent coral bleaching events and subsequent recovery of affected sites.

Methods

Coral bleaching

Rapid assessment surveys were carried out at 26 sites around the island during June and July 2007 (Fig. 1). Sites were identified on the reef flat, reef slope and on the patch reefs within the lagoon.

Surveys were carried out using snorkelling techniques by a team of 2 - 3 personnel. Surveys on the reef flat were carried out at high tide so that surveyors could swim into the lagoon over the reef front and thus assess bleaching on the reef slope down to a depth of 5 – 6 m. Surveyors swam across the reef area for two minutes and then stopped and recorded the occurrence of bleaching within an area of approximately 5m x 5m. This was repeated to give a total of 20 observations per site. During each observation for each coral genus observed, the abundance of the genus and the percentage of colonies of that genus that was recently dead (still standing, but covered in a thin layer of turf algae), bleached (pale to completely bleached) and healthy was recorded on a 6-point semi-quantitative scale (0 = 0 %; 1 = 1 – 10 %, 2 = 11 – 30 %, 3 = 31 – 50 %, 4 =

51 – 75 % and 5 = 76 – 100 %) (Turner et al. 1999). The bleaching values for each genus were then averaged and multiplied by the abundance score for that genus to give a bleaching score for each coral genus observed. These values were then averaged to produce a mean bleaching value for each site.

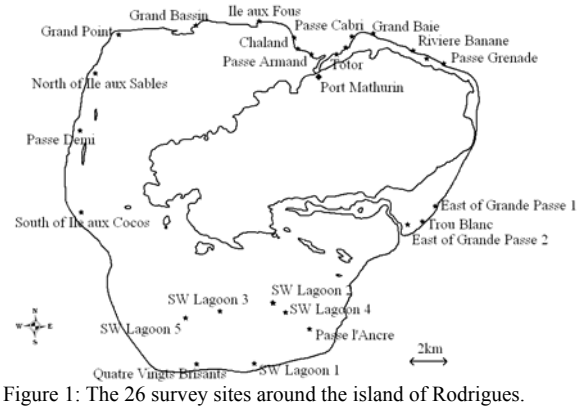

Figure 1: The 26 survey sites around the island of Rodrigues.

National Oceanic and Atmospheric Administration (NOAA) Sea Surface Temperature (SST) Hotspot charts and meteorological data (temperature, SST and sunshine hours) obtained from the Mauritius Meteorological Services were studied to identify annual large-scale variations in environmental conditions. Smaller scale spatial variations were investigated using data collected by Shoals Rodrigues. Continuous temperature loggers were placed on the reef flat at 3 sites in the north of Rodrigues (Totor, Grand Bassin and Ile aux Fous) between March 2006 and December 2007 and recorded SST every hour. Data on current speeds around the lagoon, collected using drogue buoys in 2002, were also analysed.

Recovery from bleaching

Additional detailed surveys were undertaken at three sites, which had been worst affected by the 2002 and 2005 bleaching events: Totor, Passe Armand and Ile aux Fous. All surveys were undertaken on the reef flat at depths of approximately 1 m. Three 20 m transect lines were laid parallel to the reef edge and separated by a distance of 10 m. Benthic community was assessed along each of the transects using the line intercept transect method (English et al. 1994). The abundance of coral recruits (colonies <10 cm in diameter) was also assessed by counting all of the colonies within thirty 0.25 m^2 quadrats randomly placed along the transects. These data were compared with similar surveys undertaken in 2003 (Hardman 2004) and 2006 (Stampfli 2006) to assess recovery over time.

Results

Coral bleaching

Recent coral mortality was observed on one patch reef in the southern lagoon (SW Lagoon 5). At this site, 100% of branching and tabular *Acropora* spp. were dead and had been recently colonised by turf algae. Live coral cover was <1 % and consisted of small encrusting and massive coral colonies (*Goniastrea* sp. and *Cyphastrea* sp.) as well as *Fungia* spp. Nearby patch reefs (<1 km away) were not affected by this bleaching and no other coral bleaching was observed. Indeed, at 95% of the sites surveyed during 2007, 76 – 100 % of coral colonies were healthy and showed no sign of bleaching or recent mortality.

NOAA SST charts indicate that Hotspots values were 1.0 ^oC above the climatological maximum from 16th until 26th March 2002; >1.0 for a 1-month period from 26th February until 26th March 2005 and were >0.5 from 26th January until 20th February 2007 (NOAA/NESDIS/OSDPD 2008). Meteorological data indicate that maximum sea temperatures >30 ^oC were recorded in February 2002, March 2005 and January 2007 (Fig 2). Maximum air temperatures were above the long-term mean (1971 - 2000) during January - March 2002 and 2005 and during January 2007; hours of sunshine were above the long-term mean during February 2002, January 2005 and January 2007 and wind speeds were below the long-term mean during January and February 2002, March 2005 and February and March 2007.

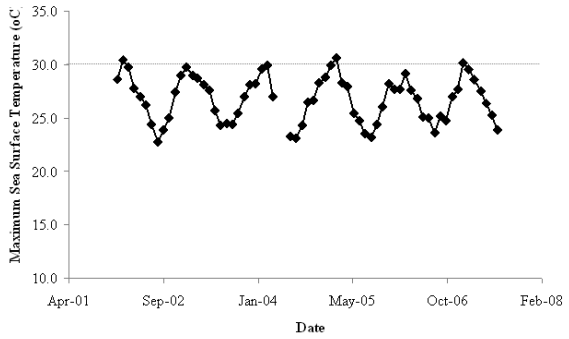

Figure 2: Sea surface temperature (SST) data for the Rodrigues lagoon from January 2002 – July 2007. Data provided by the Mauritius Meteorological Services.

Data from the continuous temperature loggers suggest that there are small-scale variations in SST around the lagoon and mean SST was significantly higher at Passe Cabri that at Grand Bassin and Ile aux Fous (1-way ANOVA, $p < 0.001$). Current speeds ranged from 0.48 m. s^{-1} in the southern lagoon to <0.1m. s^{-1} in the Port Mathurin Bay area and were significantly higher in the southern lagoon (mean: 0.27 m. s^{-1}) compared to the northern lagoon (mean: 0.18 m. s^{-1}) (T-Test, $p < 0.05$).

Recovery from bleaching

In 2007, the sites at Totor and Passe Armand were dominated by turf algae (>60 %) with low live coral cover (<15 %). In contrast, Ile aux Fous had a live coral cover of 21% and was dominated by coralline algae (49 %). The abundance of coral recruits varied from 2.0 ind. m^{-2} at Totor to 13.2 ind. m^{-2} at Ile aux Fous.

Passe Armand and Totor showed no change in live coral cover over time (Fig 3). The percentage cover of turf algae increased from 57 % in 2003 to 68 % in 2007 at Passe Armand and from 34 % in 2006 to 65 % in 2007 at Totor. At Ile aux Fous, live coral cover increased from 14 % in 2003 to 21 % in 2007; rubble fell from 35 % in 2003 to 0 % in 2007 and coralline algae increased from <1 % to 49 %.

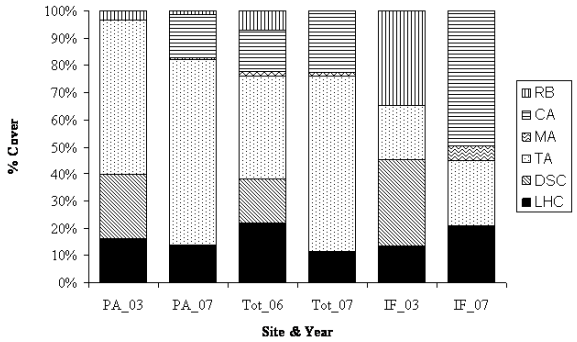

Figure 3: The percentage cover of 6 benthic categories at Passe Armand (PA), Totor (Tot) and Ile aux Fous (IF) during 2003, 2006 and 2007 assessed using the line intercept transect method.

Discussion

Coral bleaching

The data from 2002, 2005 and 2007 highlight small-scale variations in coral bleaching around Rodrigues. In both 2002 and 2005, sites in the north and west of the island were most severely affected. In 2002, 50 – 100 % of branching and tabular *Acropora* spp. died at 5 sites in the north of the island, whereas sites in the south and east were either healthy (no bleaching) or suffered from partial bleaching on the upper surfaces of branching colonies only (Hardman et al. 2004). In 2005, >50 % of coral colonies bleached at 10 sites in the north and west of the island and coral mortality occurred at 1 site, whereas bleaching in the south and east was mild and patchy (Hardman et al. in press). This study found that coral bleaching had occurred on just 1 patch reef in the southern lagoon, whereas sites <1 km away did not suffer from bleaching-related mortality. Coral bleaching was most widespread during 2005, but subsequent mortality was greater during 2002. In all cases, coral bleaching occurred at depths of <2 m and branching and tabular *Acropora* spp. were most severely affected. Previous studies have also shown that this genus is the most susceptible to coral bleaching and subsequent mortality (e.g. Brown and Suharsono 1990; Edwards et al. 2001; McClanahan 2004).

Data from NOAA and the Mauritius Meteorological Services suggest that conditions were conducive to coral bleaching during 2002, 2005 and 2007. SST data indicates that 30°C may be the threshold for coral bleaching in Rodrigues, as this was recorded during February 2002, March 2005 and January 2007, but not in years when bleaching did not occur. This is higher than the threshold of 27.5°C recorded in Mauritius (McClanahan et al. 2005), highlighting geographical variations in bleaching response. During February 2002, high air and sea temperatures, high hours of sunshine and low wind speeds all combined to promote heating of shallow water and increased solar penetration and thus explains the more severe bleaching observed during 2002. Although temperatures were high, sunshine hours were below average during March 2002 and wind speeds were above average during January 2007. Furthermore, in 2007, temperatures fell below average in February and March and February had very low cloud cover accounting for the limited bleaching that was observed during that year. This indicates that a combination of climatic factors, not just high temperature, is required for widespread coral bleaching to occur (e.g. Brown and Suharsono 1990).

Data from Shoals Rodrigues indicates small-scale variations in environmental conditions within the lagoon. SST varied significantly at sites 3 – 5 km apart and was found to be highest at the most inshore, sheltered site of Totor and lowest at the more exposed site of Grand Bassin. Totor was one of the worst affected sites in 2005 where 31 – 50 % of coral colonies bleached (Hardman et al. in press) whereas, although bleaching occurred at Grand Bassin in both 2002 and 2005, it was less severe (Hardman et al. 2004; in press). Current speeds were lower in the north of the lagoon than the south, suggesting that the decreased water flow may have increased the severity of coral bleaching at sites in the north and west of the island during 2002 and 2005. Environmental stress to corals has been shown to be reduced with high water flow whereas in areas of decreased water flow, oxygen accumulates within the coral tissues, resulting in oxidative stress and an increased severity of bleaching (Finelli et al. 2006). In contrast, McClanahan et al. (2005) show that in Mauritius, coral bleaching was positively associated with high water flow, with the most severe bleaching occurring on windward sites with the highest current speeds. They suggest that corals living in stable, less environmentally stressed environments such as these are more susceptible to bleaching increases. This may explain the bleaching in 2007, which occurred on the

southern patch reef only, a site with the highest current speeds, which had not exhibited bleaching in previous years.

Recovery from bleaching

Recovery from the bleaching events also showed small-scale variations with sites <5 km apart demonstrating different responses. Totor and Passe Armand have shown limited recovery 2 and 5 years respectively after suffering from bleaching-induced mortality and sites were dominated by turf algae with low live coral cover. In contrast, Ile aux Fous showed an increase in live coral cover, a high cover of coralline algae and the highest abundance of new coral recruits, indicating more successful recovery. Totor and Passe Armand are inshore sites (500m offshore), situated within the sheltered Port Mathurin Bay. These sites are subjected to high sedimentation, especially following periods of heavy rainfall (Hardman 2004), increased nutrient input (Shoals Rodrigues unpublished data) and human impacts such as trampling damage from octopus fishers.

The variation in recovery rates is therefore likely to be as a result of these additional human impacts. Lambo and Ormond (2006) also suggest that in Kenya, coral bleaching, sedimentation and fishing pressure have combined to result in further declines in live coral cover following the 1997/1998 coral bleaching event. Totor also has a high abundance of the bioeroding sea urchin, *Echinometra mathaei* (Hardman et al. in press) and bioerosion rates may counteract up to 150 % of the total calcification at this site. This high level of bioerosion suggests that if reefs continue to be affected by coral bleaching then there may be a loss of the coastal protection function of the reef.

Although Rodrigues escaped the mass bleaching event of 1997/1998, coral bleaching events have occurred in 2002, 2005 and 2007 causing locally severe mortality. It has been predicted that coral bleaching events will occur annually by 2030 - 2050 (Hoegh-Guldberg 1999) and Sheppard (2003) estimates that reefs in the Indian Ocean will be affected every five years by 2010 - 2025, becoming ecologically extinct within the next 20 – 40 years. If coral bleaching events continue to occur on such a frequent basis, then this will affect the integrity of the reef structure causing breaks in the reef's protective barrier, leaving these areas vulnerable to wave action and resulting in increased coastal flooding and storm damage with loss of coastal habitats and significant damage to coastal properties.

Rodrigues is a very isolated island, which receives a limited larval supply, suggesting that the reefs rely on larval retention and self-seeding for population recovery. Although McClanahan et al. (2005) suggest that the lack of bleaching in 1997/1998 and the local climatic conditions mean that reefs in this region may be more resilient to temperate anomalies, the limited larval supply inhibits recovery, making reefs more susceptible to climate change-driven reef degradation (Graham et al. 2006). These inshore reefs, already affected by human impacts, are therefore at risk of suffering a phase-shift to an algal dominated community with the associated loss of structural complexity. Rodrigues is highly reliant on its coral reef resources for food, employment and coastal protection and a loss of reef structure could have very serious consequences for the future of this small island.

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Impacts of non-point source sewage pollution on Elkhorn coral, *Acropora palmata* (Lamarck), assemblages of the southwestern Puerto Rico shelf

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Abstract. Non-point source sewage pollution represents a major threat to coral reefs. Impacts are typically associated with chronic eutrophication, water turbidity, and microbes potentially pathogenic to corals. Sewage pollution can produce variable system- and species-specific responses, as well as cascading direct and indirect effects, that could result in major long-term phase shifts in coral reef benthic community structure. This study was aimed at characterizing the ecological condition of eight shallow-water (<5 m) Elkhorn coral (*Acropora palmata*) assemblages located across a non-point source sewage pollution gradient along the southwestern Puerto Rico shelf. Non-point source pollution was a key stressor structuring local coral reef communities. Long-term phase shifts have favored dominance by macroalgae and non reef-building taxa at inshore locations under chronic pollution. Non reef-building taxa correlated with fecal pollution indicators. *Acropora palmata* and crustose coralline algae (CCA) are dominant at offshore remote reefs. Coral reef degradation is already beyond the point of recovery at most inshore habitats. Coral reef communities within local Marine Protected Areas were also undergoing significant degradation as a result of variable impacts, including non-point source sewage pollution. There is a paramount need to implement adequate management measures to prevent further water quality degradation across the region.

Key words: *Acropora palmata*, Coral reefs, Non-point source sewage pollution, Puerto Rico

Introduction

Marine non-point source sewage pollution is a major cause of concern in coral reef communities. Negative sewage impacts have been mostly associated to eutrophication and turbidity (Pastorok and Bilyard 1985; Cloern 2001). Kaczmarsky et al. (2005) also documented a high prevalence of Black Band Disease and White Plague-Type II in coral colonies exposed to sewage. Coral survival rates (McKenna et al., 2001), as well as reef-building activity (i.e., skeletal extension rates), are highly susceptible to sewage impacts, although effects seem to be species-specific (Tomascik and Sander 1985; Spencer-Davies 1990). Sewage impacts often result in a combination of system- and species-specific responses, as well as cascading direct and indirect effects that could result in major long-term phase shifts in benthic community structure, favoring dominance by fleshy macroalgae and non reef-building taxa. Such phase shifts could be irreversible in long-term scales (Knowlton 1992; Hughes 1994). Sewage-associated eutrophication impacts can also result in an accelerated reef decline often due to a combination of synergistic impacts, mostly from sedimentation and turbidity (Meesters et al. 1998; Szmant 2002), as well as to recurrent pulses of increased biological oxygen demand and declining dissolved oxygen concentration that can create a hypoxic condition in coastal waters (Desa et al. 2005).

Sewage impacts can produce a major decline in the socio-economic value of coral reefs and associated communities due to the loss of ecological services (i.e., coastal protection, sinkhole of greenhouse gases, food-protein production, source of bio-active compounds), and reef aesthetics (i.e., SCUBA, snorkeling, educational excursions). Declining reefs may also represent a permanent phase shift to fisher community livelihoods and a loss of cultural heritage. Sewage has been previously implicated in coral reef degradation in Puerto Rico (Goenaga and Boulon 1992; Hernández-Delgado 2000, 2005). In spite of that, there is very limited information regarding the impact of non-point source sewage pollution in benthic community structure

in coral reefs, particularly in highly susceptible species such as Elkhorn coral, *Acropora palmata*.

This study aimed at characterizing the ecological condition of eight shallow-water (<5 m) *A. palmata* assemblages located across a non-point source sewage pollution gradient along the southwestern Puerto Rico shelf. This species constitutes one of the most significant reef-builders in the Atlantic, but their populations have declined by an estimated 97% during recent decades through the region, including Puerto Rico, due to a combination of natural and anthropogenic factors (Hernández-Delgado 2000; Weil et al. 2003). Elkhorn coral was designated in May 2006 as a threatened species under Endangered Species Act 4d rule.

Materials and methods

Studies were conducted at eight coral reefs located along a non-point source sewage pollution gradient across the southwestern Puerto Rico shelf (Fig. 1). Sampling was limited to shallow reef assemblages (<5 m).

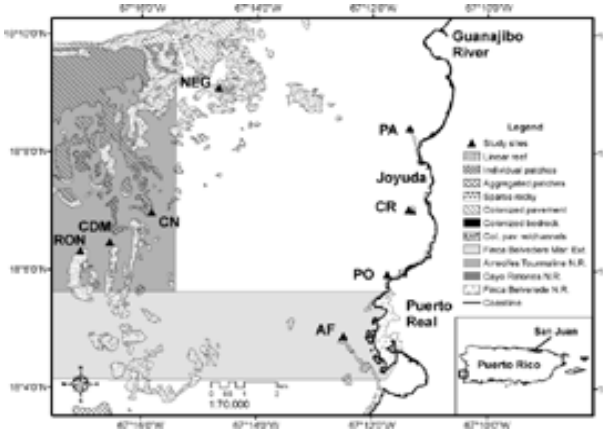

Figure 1. Coral reef study sites: Arrecife Fanduco (AF), Punta Ostiones (PO), Cayo Ratones (CR), Punta Arenas (PA), Arrecife El Ron (RON), Cayo del Medio (CDM), Corona del Norte (CN), and El Negro (NEG).

Sites were arranged into two different geographic regions (inshore, offshore). Inshore reefs (<0.5 km) included AF, PO, CR, and PA (see Fig. 1 for acronyms). Offshore sites (>0.5 km) included RON, CDM, CN, and NEG. Sites were further arranged within two management categories (MPA, non-MPA controls). MPA sites included RON, CDM, CN, CR, and AF. The first three lie within the Tourmaline Natural Reserve. Cayo Ratones is located within the Cayo Ratones and Adjacent Waters Natural Reserve, while Arrecife Fanduco is located within the Finca Belvedere Natural Reserve Marine Extension. These are managed by the PR Department of Natural and Environmental Resources (DNER). Non-MPA control sites included NEG, PO, and PA.

Six replicate 10 m-long linear transects were haphazardously sampled at each site in February 2006. Five replicate non-overlapping 1 m^2 quadrats were haphazardously photographed along each transect using digital photography. Images were corrected for brightness and contrast, and analyzed using CPCE 3.4 software (CPCe, Kohler and Gill 2006), FL. Twenty replicate randomly-generated counting points were projected over each image and used to quantify the proportion of benthic categories. Hard coral (scleractinian + hydrocoral) data was also used to calculate coral species diversity index (H'n) and evenness (J'n).

Community parameter data were analyzed by means of one-way analysis of variance (ANOVA) to test the null hypotheses of no significant site, geographic location, and management regime effects in coral reef benthic community parameters. Significant differences were identified using Tukey's test for comparisons of means. Relationships between benthic community parameter data and data about water microbiological and physical quality obtained from Bonkosky et al. (2008) were analyzed by means of a Pearson correlation analysis. Data on coral species richness and colony abundance were √-transformed, and data on the proportion of % benthic components cover were *arcsin*-√-transformed to reduce variance (Zar 1984).

Community matrices were analysed in PRIMER 6.0 (Clarke and Warwick 2001). Hierarchical clustering usin the Bray-Curtis similarity and group average linkage method (Bray and Curtis 1957) as well as non-metric multidimensional scaling (MDS) and principal components analysis (PCA) were used. Spatial variation was tested using one-way ANOSIM (analysis of similarities). Interaction effects were tested using a two-way crossed ANOSIM. Key taxa responsible for spatial variation were determined using SIMPER and PCA.

Results

Site effects

Mean coral species richness per transect was significantly higher (p=0.0035) at CN (Table 1; Fig. 2). Significantly lower coral species richness occurred at reefs subjected to hurricane disturbance and macroalgal overgrowth due to Guanajibo River influences (i.e., RON), and at inshore reefs subjected to frequent pulses of non-point source pollution. Colony abundance showed a highly significant (p<0.0001) gradient that followed non-point source pollution influences, with a mean higher abundance at offshore sites (Fig. 2). Percent hard coral cover and % *A. palmata* also followed a similarly significant trend (p=0.0004; p<0.0001) (Fig. 3). Percent cover of threatened Elkhorn

coral, *A. palmata*, followed a similar trend with a highly significant (p<0.0001) abundance at offshore remote sites. Living elkhorn corals were completely absent from PA, PO, and AF. These locations were subjected to chronic pulses of non-point pollution and high turbidity.

Table 1. Summary results of one-way ANOVA analysis of coral reef community parameters among sites (d.f.= 7, 33), between geographic locations (d.f.= 1, 39), and between management regimes (d.f.= 1, 39). NS= not significant

Parameter	Sites p	Loc. p	Management p
Species richness	0.0035	NS	NS
Colony abundance	<0.0001	<0.0001	NS
% Coral	0.0004	0.0001	NS
% *A. palmata*	<0.0001	0.0001	0.0462
H'n	NS	NS	NS
J'n	NS	NS	NS
% Octocoral	<0.0001	0.0012	NS
% Sponges	0.0123	0.0012	NS
% Zoanthids	<0.0001	NS	0.0475
% Macroalgae	<0.0001	<0.0001	NS
% CCA	<0.0001	<0.0001	NS
% Cyanobacteria	<0.0001	NS	<0.0001
% SPR	0.0003	0.0194	NS

Percent octocoral cover resulted significantly higher (p<0.0001) at AF and PO, and significantly lower at CDM and NEG. There was a major difference in species composition among sites, with *Pseudopterogorgia* spp., and *Plexaura* spp. being dominant at inshore polluted sites, and *Gorgonia* spp. being more abundant at offshore sites. Sponges were significantly (p=0.0123) more abundant at CDM. Zoanthid *Palythoa caribbaeorum* showed a wide geographic distribution, but at low benthic dominance. *Zoanthus sociatus* was significantly dominant at PA (p<0.0001).

Percent macroalgal cover showed a highly significant (p<0.0001) % cover under turbid and hypertrophic conditions at CR and PO (Fig. 4). Offshore reefs exhibited moderate abundance of macroalgae possibly as a combination of occasional

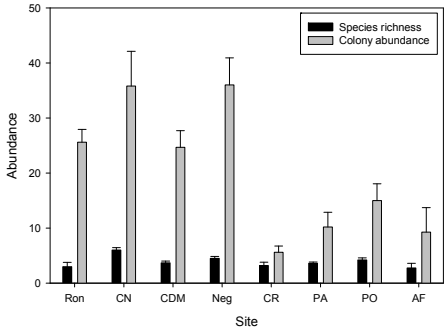

Figure 2. Hard coral species richness and colony abundance.

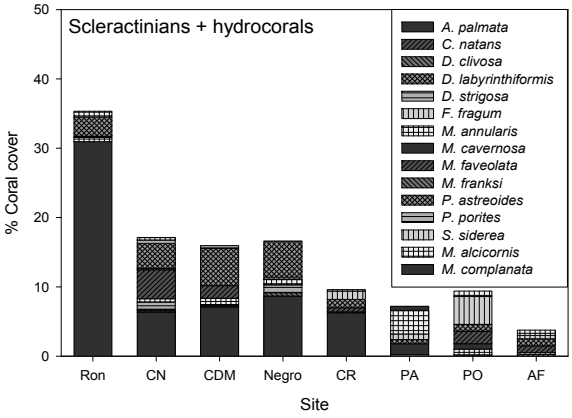

Figure 3. Percent scleractinian and hydrocoral cover.

pulse impacts by Guanajibo River plume and overfishing that has partially depleted large herbivore fish guilds (Hernández-Delgado, pers. obs.). Percent CCA cover was significantly higher (p<0.0001) at offshore remote sites. Mean lower CCA values coincided with dominance by macroalgae and other non reef-building taxa and were observed at inshore polluted sites. Percent cyanobacterial cover resulted significantly higher (p<0.0001) at NEG (Fig. 4), suggesting potential eutrophication pulses associated to the Guanajibo River plume and tidal flushing from polluted Mayagüez Bay. Recently dead corals (RDC) consistently accounted for 3-4% of benthic percent cover of non-living categories at offshore sites, and fluctuated from 1 to 5% at inshore reefs. RDC were largely attributed to the 2005 Caribbean-wide mass coral bleaching event that was followed by extensive coral mortalities until at least mid-summer of 2006 (Hernández-Delgado et al., unpub. data). About 50% of the sampled sites still showed bleached coral colonies at the moment of sampling, including AF (6%), CN (8%), CR (15%), and PO (17%). Further, 5% of the coral colonies at PO and AF were infected by white plague-like disease/syndrome conditions similar to the one that caused massive mortalities across the northeastern Caribbean reefs.

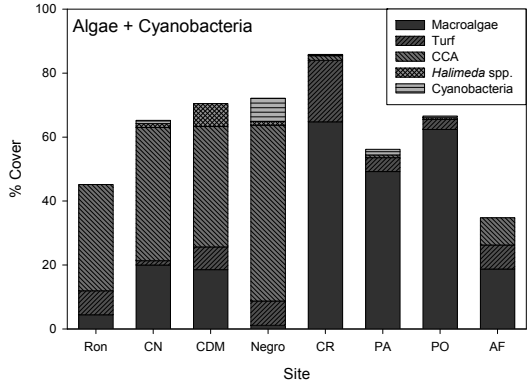

Figure 4: Percent algal and cyanobacterial cover.

Spatial patterns among sites

Two basic clustering patterns of the benthic community structure emerged from MDS analysis at the 50% community similarity cutoff level between inshore and offshore sites (Fig. 5). Four clusters emerged at the 60% cutoff level. Such patterns resulted from macroalgal, octocoral and zoanthid dominance at inshore reefs, and from coral and CCA dominance at the offshore reefs. Spatial patterns of benthic community structure were significantly different among sites (p=0.0002) (Table 2). CCA explained most of the spatial variation among sites (28.6%), closely followed by macroalgae (25%). *A. palmata* explained 14.3% of the variation,

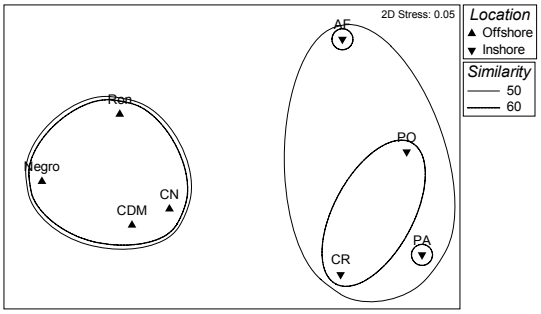

Figure. 5. MDS analysis of the community structure of shallow-water coral reef benthic communities among sites.

while *Pseudopterogorgia* spp. and *Zoanthus sociatus* explained 10.7% of the variation, each one. Spatial patterns were further suggested through PCA analysis, with *A. palmata* and CCA explaining most of the variation at offshore sites. Macroalgae and *Zoanthus sociatus* explained most of the variation at inshore sites, while *Pseudopterogorgia* spp. explained most of the variation at AF.

Spatial patterns between geographic locations

Offshore coral reefs showed significantly higher coral colony abundance (p<0.0001), % coral cover (p=0.0001), and % cover of *A. palmata* (p=0.0001)

Table 2. Results of ANOSIM test for significant differences of coral reef epibenthic community structure. Data was √x-transformed. Based on 5,000 permutations. NS= not significant.

Parameter	Global R	*P* value
One way ANOSIM		
Site	0.725	0.0002
Geographic location	0.875	0.0269
Management regime	0.052	0.3710 NS
Two way crossed ANOSIM		
Site x Location	0.512	0.0012
Site x Management	0.480	0.3333 NS
Location x Management	0.097	0.1300 NS

(Table 2). Also, sponges (p=0.0012), CCA (p<0.0001) and Sand/Pavement/Rubble (SPR) (p=0.0194) were significantly higher in offshore reefs. Octocorals (p=0.0012) and macroalgae (p<0.0001) were significantly higher at pooled inshore locations. Coral reef benthic community structure was significantly different (p=0.0269) between geographic locations (Table 2). CCA explained 14.3% of the variation between locations, followed by macroalgae (10.9%), *A. palmata* (7.5%), *Pseudopterogorgia* spp. (6%), and *Plexaura* spp. (4%). There was a significant site x location interaction (p=0.0012).

Spatial patterns between management regimes

Most parameters showed no significant differences between MPAs and non-MPA sites (Table 2). One exception was % cover in *Acropora palmata* that showed significantly higher % cover within MPA sites (p=0.0462), particularly, within Tourmaline Natural Reserve. Percent zoanthid cover (p=0.0475) and % cyanobacteria (p<0.0001) resulted higher within non-MPA control sites. Coral reef benthic community structure showed no significant difference between management regimes (Table 2). CCA explained 11.2% of the variation in benthic community structure between management regimes, followed by macroalgae (9%), *A. palmata* (8.6%), *Pseudopterogorgia* spp. (6%), and *Zoanthus sociatus* (4%). These results revealed that impacts of non-point source pollution have not discriminated between management regimes, further implying that pollution pulses can potentially affect widespread geographic areas and that current management measures have had no impact on water quality.

Relationship of coral reef community parameters and non-point source fecal pollution

A Pearson correlation analysis revealed several significant correlation patterns with microbial parameters obtained from a simultaneous study by Bonkosky et al. (2008). Zoanthids and cyanobacteria (r>0.95, p<0.01) were strongly correlated with enterococci counts, suggesting their dominance under hypertrophic, fecal-polluted conditions. Macroalgal cover was significantly correlated (r=0.94, p<0.02) to the frequency of *Bacteroides* GB32 molecular marker. *Bacteroides* is an anaerobic microorganism that grows in human and other warm-blooded animals gut. Therefore, its presence in environmental samples reveals a recent fecal pollution event. Macroalgae showed a marginally significant relationship (r=0.87, p=0.053) with *Bacteroides* molecular marker HF183. This probe is specific for human-derived fecal pollution. This suggests that inshore coral reefs were

subjected to non-point source sewage pollution, mostly from human sources.

Discussion

There is unequivocal evidence that coral reefs along a significant portion of the southwestern Puerto Rico shelf are being significantly impacted by non-point source sewage pollution, mostly from human origin (Bonkosky et al. 2008). The historical combined and cumulative impact of natural factors (i.e., hurricanes), with long-term pollution pulses, and other potential anthropogenic reef degrading factors, such as sedimentation pulses and overfishing, have contributed to a dramatic phase shift in coral reef community structure. They have favored dominance by macroalgae and non reef-building taxa. Current MPA management activities have had no significant impact on the status of coral reef benthic communities. The spatial extent of non-point source sewage pollution can be frequently underestimated due to failure to detect fecal pollution or due to the lack of water quality monitoring. But recurrent pulses can be a major long-term threat to coral reefs in face of climate change and need to be addressed.

Conclusions

Most inshore coral reefs along the southwestern Puerto Rico shelf have significantly degraded at such a magnitude that recovery may never occur in a human time scale. Stronger efforts are needed to prevent further degradation of remote reefs through the region. There is an immediate need to implement a sound management strategy to reduce and/or prevent non-point source sewage pollution impacts in coral reefs before we witness an ecological and socio-economic collapse. Controlling and managing pollution will require a definite strong political will and commitment in the government of Puerto Rico aimed at establishing stringent controls over land use patterns and over land-based pollution. Failing to recognize and manage non-point source sewage pollution may result in further loss of remote reef systems along the Puerto Rico shelf that are already showing the early signs of degradation.

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Session 18

Coral reef habitat around New Providence Island, Bahamas

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Abstract. In July 2006, the Academy of Natural Sciences of Philadelphia organized an expedition to New Providence Island, Bahamas. Coral species richness and cover, and reef surface rugosity were examined at Delaport Point (DP), Green Cay (GC), and Long Cay (LC). Greatest number of coral species (27) was observed at DP2 and the fewest (14) at DP3. Rugosity was greatest at GC1 due to the spatial complexity of an *Acropora palmata* reef. Coral cover tracked well with rugosity index (RI); GC1 with an average RI of 1.7 had coral cover (20.56%) superior to the other stations. Algae were the most abundant benthic cover component: mean= 50.99 ± 25.45% (SD); stony coral cover ranged from 0.65 to 20.56%, and the mean was 6.72 ± 6.94%. Bray Curtis similarity was greatest among GC stations and transects. ANOSIM two-way test documented that replicate transects at sampling stations were not different (Global R =0.066); however, site locations were different (Global R = -0.259). SIMPER analysis showed that macro algae genera *Dictyota, Lobophora,* and *Stypopodium* were responsible for differences in the station assemblages. Taxonomic Distinctness and Variation in Taxonomic Distinctness evaluations reported that Distinctness is stable but high Taxonomic Variation may indicate community instability.

Key words: New Providence Island, Bahamas; Community structure; Taxonomic distinctness; Rugosity

Introduction

The Bahamas archipelago (260,000 km^2) extends 800 km from SE Florida to northern Hispaniola (Gerace 1988). The majority of The Bahamas is located on two shallow banks, ideal for coral reef development. Land (islands and shoals extending above the highest tides) occupies 11,400 km^2 (4.4% of the Bahamas area). The islands are low with few cases of greater than 30 m elevation. New Providence Island is situated on an east-west axis between Andros Island to the west and Eleuthra Island to the east; the islands are situated on the Great Bahamas Bank.

Engelhard (1915) reported that, of the reefs around New Providence Island, the best coral reef development was at Sandy Cay, due north of the eastern point and Goulding Cay, west of the western point of the island. Goulding Cay was the site of a multi-year coral growth-rate study (Vaughan 1915). From the east end of New Providence a chain of reefs extends to Eleuthera; reefs were also prolific in the region north of Spanish Wells (Haweis 1917; Miner 1924, 1931, 1933). Haweis published a fascinating small book about the coral reefs of Nassau, calling them "sea gardens." He noted that there were five shallow habitats (types of ocean bottom) around the Bahamas: "soft sand; flat, sandy coral rock; coral reef; feather bar; and grass." In those days, visitors could board a glass bottom motor launch at the Colonial Hotel to have a look at the reefs (Haweis 1917).

In July 2006, the Academy of Natural Sciences of Philadelphia organized an expedition to study historical sampling locations based on the Böhlke and Chaplin (1968) field notes obtained from the Academy and the personal recollection of Gordon Chaplin who participated in the original studies (Chaplin 2006). We examined coral reef habitat (coral species diversity, coral cover, and rugosity of the reefs) in conjunction with Kellogg's reef fish studies (in progress).

Material and Methods

Each site (Delaport Point [DP], Green Cay [GC], Long Cay [LC]) included three sampling stations stratified by depth (1: 1.5 to 6.1m; 2: 6.2 to 7.6m; 3: 7.7 to 15.2 m). Three 25m long transects were established at each station (Table 1).

We sampled stony coral species richness with a 20 minute long visual survey. A five mega-pixel digital camera in an underwater housing with a reference rod that positioned the camera 40 cm from the reef surface was used to capture benthic cover (N=40 images per transect); image size was approximately 0.16 m^2. Forty images provide a planar area of 6.4 m^2 per transect and 19.2 m^2 per sampling station. Benthic cover was analyzed using point count analysis (Jaap

and McField 2001; Kohler and Gill 2006). Rugosity was measured with a chain along a ten m long path at each transect (Dahl 1973).

Sites:	Green Cay (GC)	Long Cay (LC)	Delaport Point (DP)
Station 1	25°06.283'N 77°11.822'W	25°05.576'N 77°23.899'W	25°04.786'N 77°26.631'W
Station 2	25°06.292'N 77°11.857'W	25°05.539'N 77°23.253'W	25°04.472'N 77°28.210'W
Station 3	25°06.547'N 77°11.762'W	25°05.705'N 77°23.417'W	25°05.279'N 77°26.306'W

Table 1. Sampling station coordinates.

Multivariate analyses, based on benthic components (species and genera), used Clarke's (1993) non-parametric approach, implemented in the PRIMER-E 6 software (Clarke and Gorley 2006). All point-count values were square-root transformed, so that the multivariate analyses would draw on species from across the whole assemblage rather than being either dominated only by the two or three species with the highest cover or overly influenced by rare species (Clarke and Green 1988). Bray-Curtis similarities were then computed to compare transects and station samples (Bray and Curtis 1957; Bloom 1981). Finally, triangular matrices were input to non-metric multidimensional scaling ordination (MDS) (Kruskal 1964) and ANOSIM test for establishing differences between habitat or location groups (Clarke and Green, 1988; Clarke 1993). The SIMPER procedure (Clarke 1993) was used to identify species most responsible for observed differences in assemblage structure.

TAXDTEST was employed to evaluate the taxonomic distinctness of the species assemblages (Clarke and Warwick 1998; Warwick and Clarke 1998; Warwick and Clarke 2001). The procedure evaluates taxonomic distinctness (TD, Î+) of the sample, compares it to a regional pool of all species and to the variance in taxonomic distinctness (VarTD, Lambda+). The method is robust, and independent of sampling effort (Clarke and Warwick 1998, 2001; Warwick and Clarke 2001). Data were exhibited in a confidence funnel and ellipse graphics. The regional Scleractinia database includes a comprehensive 1975 study at Grand Bahama (Jaap and Olson 2000) and compiled distribution data from Atlantic, Caribbean, and Gulf of Mexico locations; it was used as a comparison with the New Providence data set. The TAXDTEST was also used for regional comparison, including the Gulf of Mexico, eastern Atlantic, Bermuda, Brazil, and Caribbean.

Results

Stony coral species richness was greatest (27) at DP2 (Table 2) and the poorest (14) at DP3. Six species (*Millepora alcicornis, Siderastrea siderea, Porites astreoides, Diploria labyrinthiformis, Diploria strigosa*, and *Montastraea annularis*) occurred at all sites and stations. *Acropora cervicornis, Scolymia lacera, Scolymia cubensis,* and *Mycetophyllia ferox* were only seen at one station.

Site and Station	RI	S	Algal cover	Coral cover
DP1	1.47	16	28.26	2.04
DP2	1.44	27	51.85	11.95
DP3	1.20	14	2.83	0.65
GC1	1.77	18	32.22	20.56
GC2	1.83	18	72.06	6.29
GC3	1.43	22	73.19	12.98
LC1	1.49	15	57.10	2.99
LC2	1.22	19	81.68	1.81
LC3	1.15	21	59.80	1.23

Table 2. Mean Rugosity Index (RI), species richness (S), algal and coral cover (%) for the New Providence stations.

Rugosity was greatest at GC1 and GC2; the complexity of the *Acropora palmata* habitat was a contributing factor at GC1 (Table 2). Stations with RI values exceeding 1.4 had the greater number of stony coral species; however, the station with the highest RI values (GC2) had fewer coral species than several other stations. Coral cover generally matches well with rugosity; GC1 with an average RI of 1.71 had coral cover that was superior to the other stations. The exception occurs at station GC2 with an average RI of 1.83 but relatively low coral cover (Table 2). Spearman rank correlation for coral cover, number of species, rugosity, and taxonomic distinctness documented mediocre correlations.

Macro algae dominated benthic cover (Table 2); however, it was quite variable, ranging from 81.68% at LC2 to 2.83% at DP3. Coral cover (principally dense thickets of *Acropora palmata*) was greatest at GC1. Sponges contributed 4.84 (DP1) to 0.31 (GC3) % cover, octocorals contributed 8.00 (LC1) to 0 (DP1) % cover, and zooanthids provided 0.09 (LC3) to 0 (several stations) %cover.

Benthic community structure characterized by species cover depicts that fidelity is greatest among the GC stations (Fig. 1); GC1 (*Acropora palmata* community) and DP1, expressed the greatest similarity of the sampling transects; DP3 transects had the greatest dissimilarity (Fig. 1).

Analysis of Similarity (ANOSIM) two-way crossed test documented the differences between location and habitat. Replicate transects at sampling stations are similar (Global R=0.066); however, the site locations are different (Global R= -0.259).

Macro algae genera *Dictyota, Lobophora,* and *Stypopodium* were most responsible for differences in the assemblages (SIMPER, one-way test). When the

algae genera were removed from the analysis, coral species and genera were responsible for the dissimilarity. *Acropora palmata* contributed 20 to 30% of dissimilarity, setting GC1 apart from other stations.

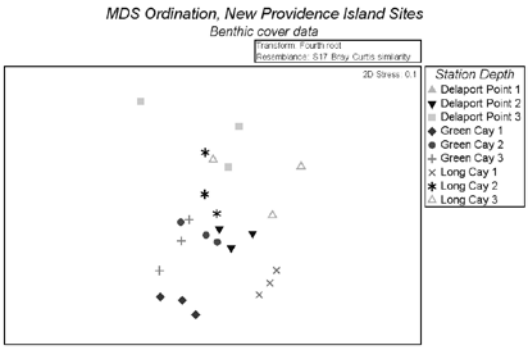

Figure 1. Multi-Dimensional Scaling (MDS) Ordination of Transects, Species Cover Attributes Used as Input for the Bray-Curtis Similarity Analysis.

Taxonomic distinctness (Delta+) values (TAXDTEST) ranged from 59.68 (GC2) to 65.28 (DP1); Lambda+ values range from 283.85 (LC3) to 614.96 (LC1). Taxonomic distinctness has not declined remarkably at these sites (Fig. 2). There is a high degree of variance in taxonomic distinctness at GC1 and LC1, likely because several of the species that occurred there were rare or nonexistent at the other sites.

Discussion

Few pre-1970 studies quantitatively assessed coral diversity and cover in the Bahamas. Photographs in these studies show evidence of coral cover; however, photos often featured the premiere attributes of the sites at that time; it is surmised from this that, typically, coral cover exceeded 30 to 40% in many locations in the Bahamas, especially in areas where *Acropora palmata* predominated.

In a study conducted in 1975 off Freeport, Grand Bahama (Jaap and Olson 2000), coral cover ranged from 1.48 to 30.28%. This was, however, for the most part in depths (30 to 70 m) that exceed the present study.

Chiappone et al. (1997) surveyed reefs in depths of 3 to 15 m along the Exuma Cays (south of New Providence Island) in 1995. Algae cover ranged from 20 to 80%, sponge cover from 10 to 40%, octocoral cover from 2 to 5%, and scleractinian coral cover from 2 to 40%. The survey reported 43 species of sponges, 29 octocoral species, and 39 stony coral species. This current study identified 32 stony coral species (Milleporina and Scleractinia) from the three sites around New Providence Island.

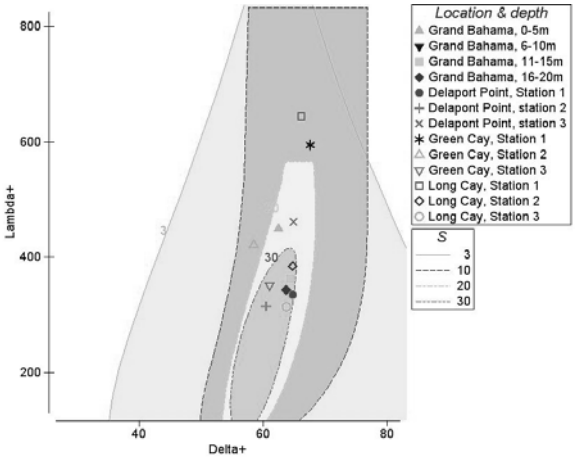

Figure 2. Taxonomic Distinctness (Delta+) and Variation in Taxonomic Distinctness (Lambda+) for the New Providence and Lucaya, Grand Bahama, Sampling Stations.

Zooxanthellate Scleractinia (ZS) distribution and taxonomic distinctiveness for the Gulf of Mexico, Florida, Bahamas, Caribbean, and south Atlantic localities are related to spatial distances: the southeastern and southwestern GMEX regions have a high degree of faunal similarity with SE Florida, the Bahamas, and the Caribbean; however, there is a low similarity with southeastern USA (North of the St. Lucie Inlet to Cape Hatteras), south Atlantic, and the eastern Atlantic (Figs. 3 & 4). Ocean current patterns within the Gulf and Caribbean provide a source of connectivity for Bahamas, Cuba, Florida, and Mexico. The cluster pattern (Fig. 3) is consistent with connectivity in the region and isolation from distant locations.

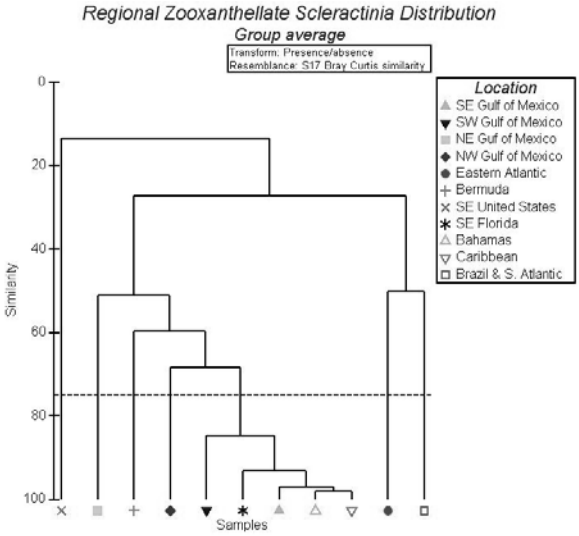

Figure 3. Cluster diagram of the distribution of zooxanthellate Scleractinia in the Atlantic Ocean and Gulf of Mexico; presence or absence of species at these locations. Bray Curtis Similarity coefficient and group average sorting.

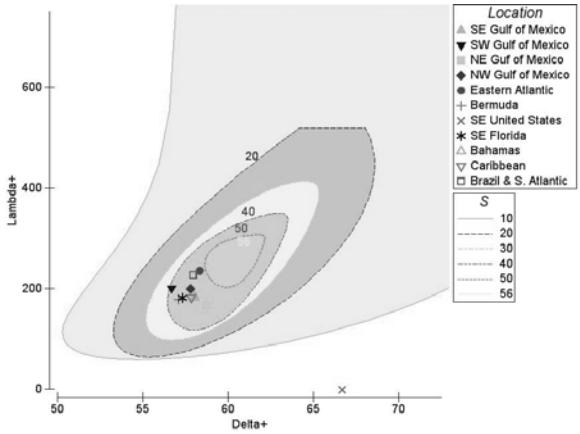

Figure 4. Taxonomic Distinctness (Delta +) and Variation in Taxonomic Distinctness (Lambda+); regional comparison of zooxanthellate Scleractinia.

We interpret the high VarTD to be the result of a reduction in certain habitats that are obligatory for some species at some sites, the abundance and competition by algae for space, and the result of tropical storm disturbances in 2004 and 2005. A comparison of the New Providence Island Site VarTD with regional VarTD (Figs. 2 & 4) shows relatively lower values for VarTD at the regional level. TD distinctness and variation (Fig. 4) in contrast to the cluster analysis (Fig. 3) shows that the zooxanthellate Scleractinia do not exhibit a large range of Delta+ and Lambda+ in the remote locations, such as Brazil, Azores, and Bermuda. This is due in part to very few endemic species and to the wide distribution of many species in the region.

Decline in coral reefs around New Providence Island may be caused by runoff and groundwater seepage from the surrounding highly populated areas. Runoff carries nutrients, metals, pesticides, chemicals, and biocides, all of which are detrimental to the vitality of the coral reef communities. We noted that there are virtually no attached organisms at DP1 and DP3 sites, which are in close proximity to densely populated urban areas and are situated near a channel facilitating transport from the shoreline. Green Cay sites are located a moderate distance from the urban developments on New Providence, and the typical epibenthic reef flora and fauna are common.

Nutrient enrichment often results in increases in macroalgal growth, particularly in areas near coastal development and increased sewage outflow (Smith et al. 1981; Birkeland 1988; Pastorak and Bilyard 1985). Macro algae were the most prevalent biotic cover component at all nine of the sampling stations. We also suspect that the large algae population is related to a decrease in herbivores (e.g., fish and *Diadema antillarum*).

Comparisons of reefs in Montagu Bay (New Providence Island) with 1943 aerial photos documents that startling declines in stony coral cover have occurred over the last half century (Sullivan Sealey 2004). Patch reefs in the bay decreased from 214 to 133 in 52 years. Aerial photographs also indicate that many coastal construction projects were undertaken between 1943 and 1995, significantly altering the coastline and, most likely, increasing the physical destruction of reefs through increased sedimentation and turbidity. Bahamian reefs were recently listed as over 60% degraded, trailing Jamaican and Panamanian reefs in total loss (Pandolfi et al. 2003). The Bahamas have made a commitment to conserve 20% of their coral reef ecosystems in the form of no-take areas or marine protected areas (Pandolfi et al. 2003), although no timeline has been directly specified. No-take reserves and marine protected areas have been shown to be effective mitigation and replenishment measures, and it is important that they are established in heavily populated areas such as New Providence Island, where unsustainable development has already led to significant declines in fish, corals, and other benthic invertebrates. These measures are limited, however, in their ability to combat the effects of degraded water quality. The Bahamian government needs to implement a new sewage treatment system. Continued degradation of water quality will occur unless significant improvements are made in the infrastructure.

Status of reefs in the Bahamas is far better in remote areas such as Cay Sal Bank than in highly urbanized islands such as New Providence. Reef degradation around New Providence is mostly the result of urban development pressures in the coastal zone. Status and trends of mangroves, sea grasses, and coral reefs in the Bahamas is similar to other Caribbean countries where coastal resources have been used as a tourist attraction for economic stimulus. This, coupled with radical declines in *Acropora* reefs (the causes of which are enigmatic), has resulted in coral reefs that are degraded and covered with algae. Monitoring programs can determine the status and future trends of resources; however, they can only serve as an ecological warning system. Basic research, management, conservation, and social action are required to resolve the problems, be they local, regional, or global.

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Mini-symposium number 18

Comparison of Benthic Communities on Six Coral Reefs in the Veracruz Reef System (Mexico)

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Abstract. The Veracruz Reef System consists of 25 platform reefs that are split into two subgroups, northern and southern, by the influence of the Río Jamapa. Scleractinian coral cover on all reefs has declined dramatically since the 1960s. Using historical data and regression analysis, we predicted ~15% cover for the northern subgroup and no coral cover in the southern subgroup. Anegada de Adentro and Blanquilla, in the northern group, and Anegada de Afuera, Cabezo, Enmedio, and Santiaguillo, in the southern group, were surveyed during Fall 2002 with photo-transect techniques. Scleractinian cover averaged 3.2% in the northern subgroup and 6.3% in the southern. Algae, particularly turf and crustose forms, dominated reef benthic communities, with greater than 50% cover on all reefs and at all depths. Coral cover was much less than predicted in the northern subgroup and was greater than predicted in the southern subgroup. Numerous natural impacts (low salinity, heavy sedimentation, diseases, winter cold fronts, hurricanes) and anthropogenic impacts (coral mining, dredging, landfill, ship groundings overfishing, coral and shell collection, others) have undoubtedly caused much of this decline. In the southern group, declines appear to have leveled off while becoming more severe in the northern group.

Key Words: coral decline, Veracruz Reef System, southwestern Gulf of Mexico

Introduction

The coral reefs of the southwestern Gulf of Mexico lie along the western boundary of the Caribbean Biotic Province (Tropical Western Atlantic Region) (Briggs 1974, Tunnell 1988). The highly endangered Veracruz Reef System (VRS) (Bryant et al. 1998) located off the state of Veracruz, Mexico (Fig. 1) consists of 25 platform-type coral reefs. These reefs are divided into two subgoups by the influence of the Río Jamapa: the northern near the city of Veracruz and the southern near Antón Lizardo, a small fishing village. Compared to other reefs within this biotic realm, these reefs have lower stony coral diversity, primarily due to frequent climatological events and turbidity from coastal runoff. Southwestern Gulf of Mexico corals occur at the limits of their distribution limiting reef biodiversity thus anthropogenic stressors have profound effects on the ecosystem (Tunnell 1988, Nelson 1991). The combination of both natural and anthropogenic disturbances exacerbates reef impacts and reduces coral cover.

Historically, live benthic coral cover on reefs in this area has been less than 40% and on nearly all reefs coral cover has declined in recent years (Lang et al. 1998). Most of these reefs have been greatly affected by overharvesting, pollution, and recreational diving; the amount of stress and destruction depends on their proximity to urbanized areas (Tunnell 1985, 1988, 1992, Chávez and Tunnell 1993). Due to the city of Veracruz's large size and reef geographic location, the VRS has suffered the heaviest anthropogenic impacts of any reefs in Mexico (Bryant et al. 1998, Jordán-Dahlgren and Rodríguez-Martínez 2003). Coral cover has declined since the 1960's and algal cover has increased.

Figure 1: Reefs of the Veracruz Reef System, in the southwestern Gulf of Mexico, near Veracruz and Antón Lizardo, Mexico. Modified from Tunnell (2006).

The goal of this research was to assess the condition of six reefs in the VRS: Anegada de Adentro and Blanquilla in the northern subgroup and

Anegada de Afuera, Enmedio, Santiaguillo, and Cabezo in the southern subgroup. Cover of scleractinian corals on reefs in the VRS has declined by more than 60% since the 1960's (Horta-Puga and Barba-Santos 1999). Based on regressions of historic coral cover data (Kühlmann 1975, Rannefeld 1972, Secretaría de Marina 1987, Nelson, 1991, Horta-Puga 2003), we predicted ~15% cover for the northern subgroup in 2002 and no coral cover for the southern subgroup (Fig. 2).

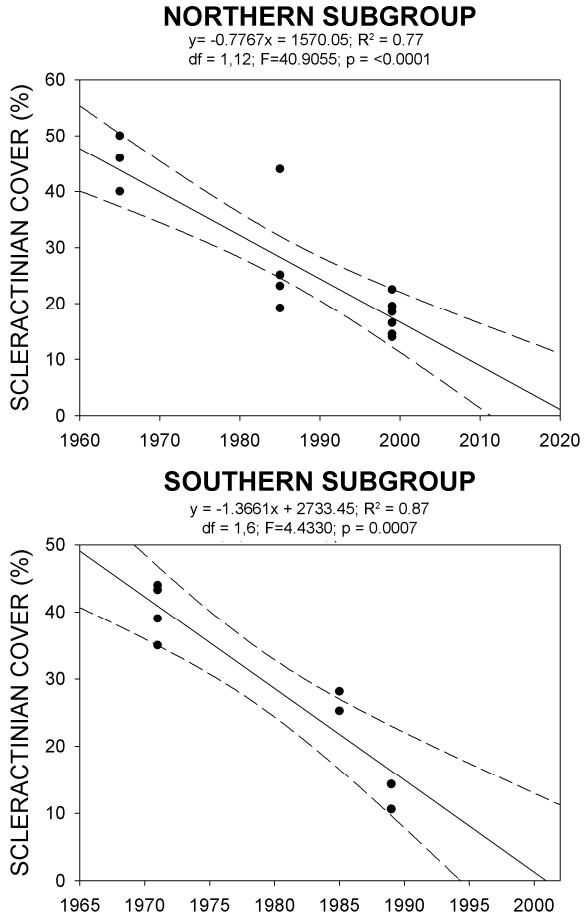

Figure 2: Trends in scleractinian coral cover in the northern and southern reef subgroups of the Veracruz Reef System, Mexico.

Methods

Each of the six reefs was surveyed using photographic techniques (Liddell and Ohlhorst 1987) at 6m, 12m, 18m, and when depth permitted, 30m. Six transects, each consisting of 12 non-overlapping photos, were completed at each depth although not all transects produced usable images. Photographs were processed to produce digital images. Each photo was projected and overlain with 144 points arranged in a uniform pattern. The coral taxon or benthic category (e.g., crustose algae, bare etc.) under each point was determined and recorded. Percent cover of scleractinian corals, algae and bare areas were calculated for each transect and averaged by reef and depth.

Results

Algae dominated benthic communities of all reefs and at all depths (Fig. 3). In the northern subgroup, coral cover averaged 3.2% and algal cover averaged 69.4%. Although very low, coral cover varied substantially with depth ranging from 1% or less at 6m and 18m on Blanquilla to the maximum of 6% at 12m. On Adegada de Adentro, maximum coral cover was also found at 12m (8%) and ranged from 0.5% at 6 m to 4% at 18m. Algal cover was greatest at 6 m on both reefs, and varied slightly with depth ranging from 58-76% on Blanquilla and 66-80% on Adentro.

In the southern subgroup, coral cover averaged 6.3% and algal cover averaged 66.2%. Average coral cover was higher in this subgroup largely due to greater cover at Santiaguillo, which ranged from 9% at 30m to 18% at 18m. Maximum coral cover on the other reefs was similar to that seen in the northern subgroup: 6% at Enmedio (12m) and Cabezo (18m), and 7% at Anedgada de Afuera (30m). Algal cover was least at Santiaguillo (50-57%) and ranged from lows of ~65% at Afuera (30m) and Cabezo (18m) to a high of 78% at Cabezo (6m).

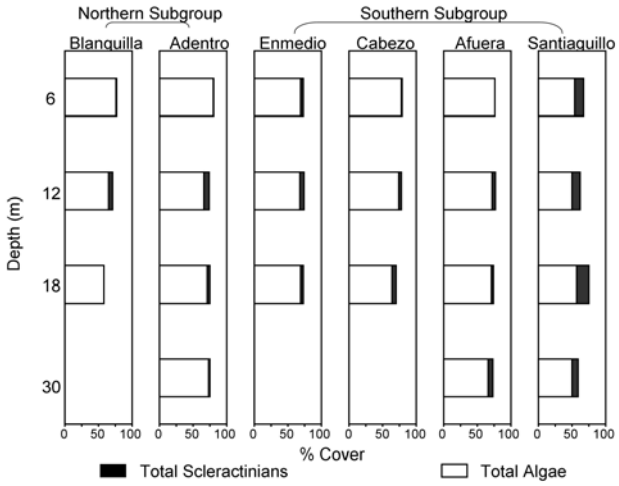

Figure 3: Average scleractinian and algal cover by depth in reefs surveyed the northern and southern subgroups of the Veracruz Reef System, Mexico. There were no 30m transects surveyed at Blanquilla, Enmedio or Cabezo.

Coral communities on the reefs surveyed were typically dominated by six species or species complexes (Table 1): *Montastraea annularis* complex, *Diploria strigosa*, *Colpophyllia natans*, *Siderastrea siderea*, *Agaricia* spp. (especially *A. lamarcki*) and *M. cavernosa*. In the northern

Table 1. Mean cover (%) of corals, algae and other taxa and categories for all depths in 2002, with standard deviation in parenthesis, on six reefs in the Veracruz Reef System, Mexico.

	Northern Subgroup			Southern Subgroup		
	Blanquilla	Anegada de Adentro	Enmedio	Cabezo	Anegada de Afuera	Santiaguillo
Algae						
Coralline red	52.4 (11.8)	32.5 (7.2)	39.1 (13.2)	66.6 (13.1)	31.5 (16.3)	46.8 (11.0)
Turf	11.1 (4.5)	23.7 (11.7)	25.7 (12.0)	1.7 (2.8)	22.3 (20.0)	0.6 (2.9)
Brown	0.7 (1.0)	5.8 (6.7)	0.4 (0.5)	1.2 (3.0)	5.9 (6.6)	5.1 (7.4)
Blue-green		3.7 (7.0)	0.1 (0.3)	<0.1	0.1 (0.3)	
Dictyota spp.	0.4 (0.7)	3.6 (3.5)	1.0 (0.8)	1.4 (1.7)	6.9 (8.0)	<0.1
Other	2.3 (1.7)	3.2 (4.0)	1.1 (1.6)	1.8 (4.0)	4.6 (5.4)	0.2 (0.5)
Scleractinians						
Acropora palmata		<0.1	0.3 (0.8)			<0.1
Agaricia spp.	<0.1	0.4 (0.9)	<0.1	0.6 (1.6)	<0.1	0.4 (0.5)
Colpophyllia natans	0.7 (1.4)	0.7 (1.7)	0.6 (1.2)	0.9 (1.7)		1.2 (2.3)
Dichocoenia stokesi	<0.1			<0.1		
Diploria strigosa	0.2 (0.3)	0.7 (1.9)	1.6 (3.2)	0.2 (0.7)	0.2 (1.2)	0.2 (0.7)
Diploria clivosa			<0.1			
Leptoseris cucullata			<0.1			0.4 (1.8)
Madracis decactis		<0.1			<0.1	<0.1
Madracis mirabilis					<0.1	
Meandrina meandrites			<0.1			
Montastraea annularis complex	0.7 (1.9)	0.5 (1.1)	1.2 (2.4)	1.0 (2.2)	0.4 (1.3)	7.9 (8.3)
Montastraea cavernosa	0.2 (0.8)	0.3 (1.3)	0.3 (0.7)	0.3 (1.0)	2.1 (0.4)	1.0 (1.6)
Oculina diffusa	<0.1	<0.1		<0.1		
Porites astreoides		<0.1	<0.1	<0.1	<0.1	0.4 (0.6)
Siderastrea radians	<0.1	<0.1	0.2 (0.6)	<0.1		
Siderastrea siderea	0.6 (1.3)	0.8 (1.8)	0.5 (1.0)	0.2 (0.9)	0.7 (1.6)	1.2 (2.5)
Solenastrea bournoni					<0.1	
Stephanocoenia michelini	0.2 (0.5)	<0.1	0.2 (0.8)	<0.1	<0.1	<0.1
Gorgonians						
Erythopodium caribaeorum	0.9 (3.3)				<0.1	
Pseudopterogorgia bipinnata					<0.1	
Hydrozoans						
Millepora alcicornis		<0.1		<0.1		
Sertularella speciosa		<0.1			<0.1	
Zoanthus spp.	<0.1	<0.1				
Stylaster roseus				0.3 (1.1)	<0.1	<0.1
Sponge	0.5 (0.5)	0.5 (0.4)	0.5 (0.5)	1.6 (1.4)	1.5 (1.5)	0.9 (0.9)
Other Fauna	0.4 (0.4)	0.2 (0.3)	0.1 (0.2)	<0.1	0.2 (0.3)	<0.1
Bare Substrate	10.2 (7.7)	3.9 (1.4)	7.3 (3.2)	8.5 (4.9)	7.4 (3.9)	17.4 (7.5)
Unidentifiable	18.2 (3.2)	19.2 (3.2)	19.5 (2.7)	13.6 (2.7)	14.7 (4.0)	15.8 (3.8)
Mean Scleractinian Coral	**2.7 (3.6)**	**3.5 (3.8)**	**5.1 (3.5)**	**3.4 (3.8)**	**3.6 (5.2)**	**12.8 (8.1)**
Mean Algae	**67.1 (8.2)**	**72.5 (6.2)**	**67.5 (4.5)**	**72.7 (6.6)**	**71.5 (7.2)**	**52.9 (9.9)**

subgroup, 11 scleractinian species were noted at Blanquilla with 14 species at Anegada de Adentro. In the southern subgroup, the most scleractinian species (16) were noted at Santiaguillo and the least at Cabezo (10). Live colonies of *Acropora palmata* were found on Enmedio as well as Anedgada de Adentro and Santiaguillo, although they were very rare on the latter two reefs.

Discussion

Coral cover and diversity in the Veracruz Reef System is naturally limited by weather, especially winter cold fronts ("nortes"), turbidity from coastal runoff, (Horta-Puga 2006, Chávez and Tunnell 2006) and their location on the tropical periphery (Withers and Tunnell 2006). Poor management and deteriorating water quality from pollution and deforestation affect the whole region, where only the most isolated reefs are generally healthy (Linton et al, 2002, Horta-Puga 2006). Multiple anthropogenic impacts, especially around large cities, have lead to declines or total losses of stony corals. As coral cover

has declined, crustose and fleshy algae have increased as space is freed up for them to colonize (Aronson and Precht 2001). The combination of the *Diadema antillarum* die-off in the 1980's and increased overfishing of herbivorous fishes (Lang et al. 1998, Tunnell et al. 2006), both of which controlled algal growth on reefs, has allowed algae to colonize the open spaces.

In this study, coral cover in the northern subgroup was less than the ~15% predicted mean and cover in the southern subgroup exceeded the mean prediction of no cover remaining. Greater average cover in the southern subgroup was primarily due to the influence of Santiaguillo, the reef furthest from shore. However, even when this reef is excluded, average cover for the southern subgroup, 4.1%, is substantially greater than predicted mean and exceeds the average in the northern subgroup.

Scleractinian coral cover has declined dramatically in both reef subgroups since the 1960's, when it averaged 40-50% overall (Kühlmann 1975; Rannefeld, 1972; Secretaría de Marina, 1987; Nelson, 1991; Horta-Puga, 2003). However, the decline to less than the predicted mean and lower 95% confidence interval cover in the northern subgroup shows that conditions deteriorated rapidly between 1999 (Horta-Puga, 2003) and 2002 (this study). On the other hand, coral cover in the southern subgroup exceeded the mean predicted, although it fell within the 95% confidence interval. This suggests that conditions causing the decline have stabilized or begun to be reversed in this area.

Acknowledgements

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A photographic method for analyzing areal chlorophyll and bleaching status of the coral *Stylophora pistillata*

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Abstract. With the increasing frequency and severity of coral bleaching events, this phenomenon has been documented and studied by scientists around the world. Methods to quantify bleaching status are based on sacrificing the samples being tested or using expensive equipment (like the diving PAM). In recent years, the performance of digital cameras in image processing has improved and they have become more affordable, thus, we used these to develop a new, economical and nondestructive way to monitor bleaching and recovery processes in coral tissues, to be used by conservationists and researchers. In the present study, we report a reliable bioassay for computerized quantification of areal chlorophyll density in corals. We used a regular digital camera and standard computer software, both inexpensive and easily available. The proposed bioassay could be used on any coral to rapidly and economically gather data on coral bleaching and disseminate them.

Key words: Coral, Bleaching, photography, chlorophyll.

Introduction

Corals and their colors

Corals and coral reef ecosystems depend on the unique symbiosis between the host animal, the coral, and single-cell algae, the zooxanthellae (Brandt 1881). These live within the coral cells, in specialized vacuoles, the symbiosomes (Trench 1971, 1974; Gates 1992).

Besides some effect of the animal's water-soluble pigments (Shibata 1969), the color of corals depends primarily on the areal concentration of the photosynthetic pigments of the zooxanthellae. Thus, it reflects the product of the density of the symbionts and their cellular pigment content, with chlorophylls being the most prominent.

Coral bleaching

In well-publicized bleaching events, corals have turned white, usually due to loss of zooxanthellae (Glynn 1996), but occasionally as the result of a decrease in algal pigmentation (paling). These bleaching episodes are triggered by a sustained increase of water temperature, occasionally acting synergistically with high light, UV radiation, eutrophication, and disease (Glynn 1990, 1993; Dubinsky & Stambler, 1996), and result in widespread colony mortality and reef destruction.

The documentation of bleaching

In order to document and monitor bleaching events around in real time, it is of great importance and urgency to develop reproducible, rapid, reliable, inexpensive, and nondestructive ways to quantify coral pigmentation. Much of the early and current reporting of bleaching events is based on diver information and qualitative descriptions (Wilkinson and Buddemeier 1994). These are insufficient and there is a need for quantifiable methods for revealing the onset of bleaching in corals, and document instances in which recovery takes place.

Past attempts to achieve this goal were difficult and tedious. Thieberger et al. (1995) used 35 mm color slides taken under standard conditions, which were then scanned in a specially designed adapter in a spectrophotometer. Several subsequent efforts at different spatial scales and resolutions were tried in order to document the status of entire reefs, usually based on expensive, custom-made, hyperspectral scanners (Elvidge 2003). However, these were inadequate for determining the advent of bleaching on a single-colony level. The use of a digital camera to determine bleaching status in corals using a color reference card has been reported recently (Siebeck at al 2006) however the present method is more convenient and precise.

Most reefs are found in remote areas and in developing countries, far from well-equipped, academic research centers. Therefore, what is needed is a means to monitor coral-bleaching events and their aftermath using straightforward, standardized procedures. Ideally, such a system – based on off-the-shelf digital underwater cameras – should be designed to be used rapidly and economically by field technicians and reserve operating personnel at hundreds of locations. The resulting digital information can then be easily communicated worldwide.

Materials and Methods

Coral fragments

Three colonies of *Stylophora pistillata* were gathered from the reef near the Interuniversity Institute, Eilat, Israel, in the Gulf of Eilat (Aqaba). Corals of 15-cm diameter were collected from artificial objects at a depth of 2.5-3.5 m. The corals were allowed to acclimate for one month in our main aquarium system under 500µ mole quanta m^{-2}. Then the corals were divided into 3-cm-long fragments and glued with Super Glue Gel (Scotch, 3M) to plastic tips (Fig. 1).

Figure 1: *Stylophora pistillata* fragments during acclimation in our main aquarium system

The Fragments had divided between controls remaining in the light and the bleaching ones in the dark for 44 days. Every two weeks 3 fragments were photographed and extracted, and from these data the calibration curve (Fig. 4) and figure 5 were obtained.

Digital photographs

Coral fragments were taken from the aquaria in a seawater-filled beaker, photographed, and immediately returned to the water to minimize air exposure, or they were processed for analyses as described below.

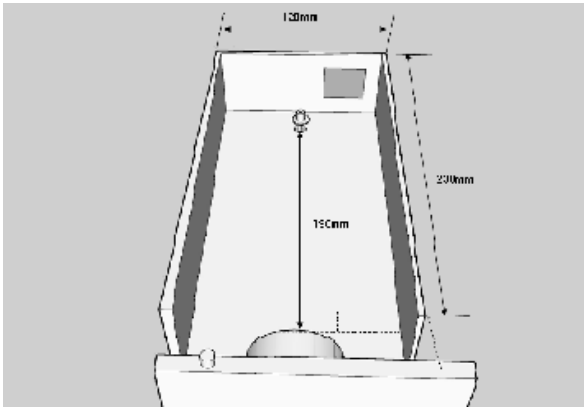

Figure 2: Camera stand sketch. The coral fragment was placed in the center of the image at 190 mm from the camera lens, with the gray plate at its right.

Digital images were taken with an Olympus 750 camera with 4 M pixel resolution. To be able to compare images, camera-to-coral distance, illumination, and state of extension of the polyp tentacles were kept constant. A gray 30□40-mm PVC plate was included as a reference (red=200, green=200, and blue=200 as a standard color reference card, see below for details) (Fig. 2) in each photograph.

We used a plastic stand to position the fragments and camera at a fixed distance and angle (Fig. 2), to the right side of the fragment position we attached the PVC plate. The distance between the camera lens and the fragment's nearest surface was 190 mm and the fragment was orientated to be at the center of the image. All the photographs were taken using the camera's internal flash. To keep the polyp tentacles fully retracted, we photographed the fragments out of the water. For an in-water procedure, one can disturb the water near the coral (by hand or pump) and cause the tentacles to retract. The gray PVC sheet was attached at the same distance from the camera as the fragment, in order to eliminate any differences in lighting since we regard its color as the standard for calculations. All pictures were taken with a shutter speed of 1/60s and aperture of f=1.8.

Picture analysis

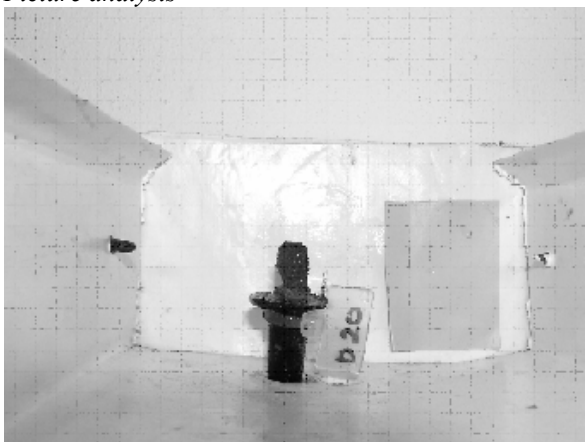

Figure 3: *A sample of the image taken by the camera. The coral fragment is in on the plastic stand with the grey reference plate to its right*.

Analysis of the digital images (Fig. 3) was done using Paint Shop Pro (Corel Corporation, Ottawa, Ontario, Canada). All images were cropped to a square of 100*100 pixels, covering an area of 7.5 by 7.5 mm live coral tissue. Likewise, 100*100 pixels of the gray sheet image were cut from the picture. For each such square, a wavelength histogram graph (including only the green channel) was produced by the appropriate Paint Shop option, and the average value of the 10,000 pixels (100*100) was calculated. This value was found to be between 0 for black and 255 for white paper.

The sample size was chosen as to include the largest possible homogenous fragment area. In the branched *Stylophora pistillata*, the average width of

the subcylindrical branches was found to be ~10 mm. The chosen size allowed us to photograph a nearly flat surface perpendicular to the camera lens. In preliminary experiments, we found that correlation improved as we increased the sample area to the maximum of 7.5 mm square.

For standardizing images and results, all readings were corrected for the gray standard as seen in Eq. 1. In this way, the lighter the coral is, the higher the resulting value.

Eq. 1 P = 255 - [Gray sheet average] + [Coral tissue average]

Thus, low P values correspond to a darker image and higher chlorophyll concentrations, the P value ranged between 100 (high) to 250 (low).

Pigment extraction and quantification

In order to calibrate the data obtained from the photographs, we used the standard methods for quantifying chlorophyll in *S. pistillata* and other corals (Dubinsky et al. 1990; Titlyanov et al. 2000; Nordemar et al. 2003). The procedure requires sacrificing the sample using the Water-Pik method (Joahnnes and Wiebe 1970; Falkowski and Dubinsky 1981; Hoegh-Guldberg and Smith 1989; Edmunds and Gates 2002) to remove the animal tissue with the zooxanthellae from the skeleton. The resulting homogenate was filtered through a 25-mm Ø glass fiber paper filter (Whatman GFC). The zooxanthellae collected on the filter were extracted in 10 ml 90% acetone/water by grinding the filter with a glass/PTF homogenizer. The resulting slurry was filtered again through the same type filter. Chlorophyll concentration was quantified spectrophotometrically with a Varian DMS 100S, according to the Jeffrey and Humprey (1975) equations from the ODs 665nm and 755nm, and normalized to sample area.

Fragment area was determined using the aluminum foil method (Falkowski and Dubinsky 1981; Hoegh-Guldberg and Smith 1989; Edmunds and Gates 2002).

Results & Discussion

Comparison the extracted chlorophyll measurements and our estimates based on analysis of the photographs of the same fragments, resulted in a logarithmic trend line (Fig. 4, Eq. 2)

Eq. 2 y = -11.41n(x) + 62.274, R2= 0.919

This proves that estimating areal chlorophyll density can be done by photographing and analyzing a digital image of a coral fragment or colony. The S. pistillata control fragments had a concentration of 7.2±0.9 chlorophyll a mg cm-2 as determined by the standard method, and 6.8±0.6 mg cm-2 estimated by our photographic method.

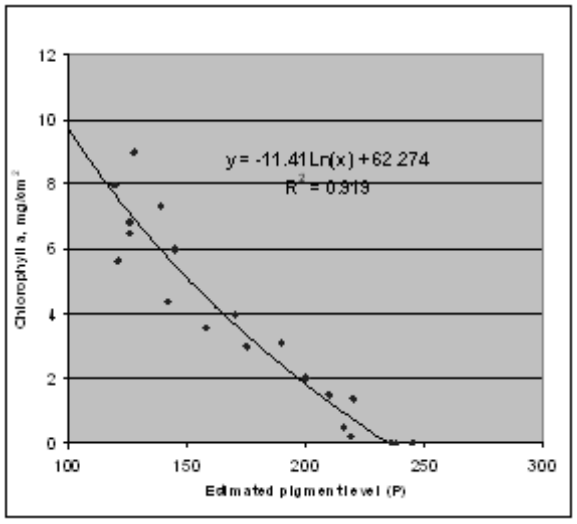

Figure 4: *The relation of estimated pigment level (P) to measured chlorophyll density. Data points are from different times during the bleaching and recovery processes*.

The usefulness of our method was further demonstrated by the results from monitoring the time course of the experimentally induced bleaching of *S. pistillata* fragments in the dark as described in the Methods section. A steady decline in chlorophyll density is seen until the 44th day, when the corals were completely bleached (Fig. 5).

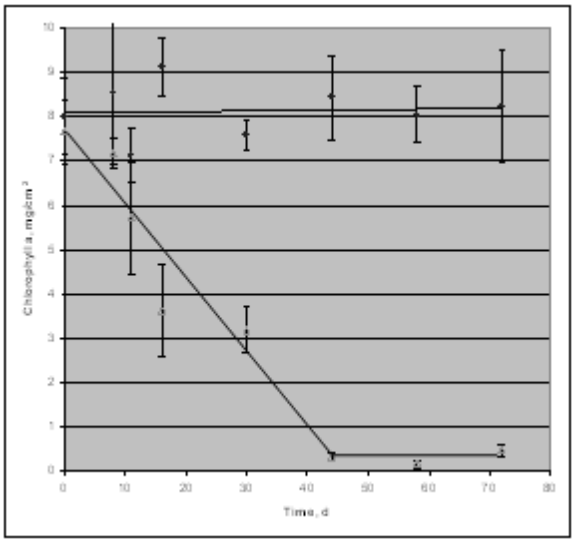

Figure 5: *Photographically estimated chlorophyll density of bleached fragments △ vs. control ● (n=5). The bleaching was complete by day 44 (as seen in the graph)*..

A calibration curve should be determined for each coral species, since the distribution of the zooxanthellae between polyp body and tentacles, the depth of algae within the calyx, and the surface rugosity, are all species-specific properties that affect

the relationship between the photographically estimated chlorophyll and its true areal concentration. Put in a different way, the color of a zooxanthellate coral, as it appears to the eye or camera, depends not only on the pigment concentration but also on its distribution over the coral surface. If the conditions under which the image is taken (distance, light, exposure, background, and camera) are changed, a new calibration curve should be generated. It is also important to take all such photographs with the tentacles in the same stage, either withdrawn or expanded, since tentacle status considerably affects the optical properties of corals (Levy et al. 2003).

The advantages of the method are obvious: once calibration is established, the tedious and error-prone tissue homogenization, pigment extraction, and area determination procedures are no longer required. Furthermore and most important, our method is totally nondestructive, rapid, inexpensive, readily computerizable and transmissible.

We believe that this bioassay can be further developed for use even on the reef scale, as long as photographic activity is done under similar conditions regarding distance and angle of the camera in relation to the reef, solar elevation, cloud cover, wave height, tidal state, etc.

By using this bioassay, one can gather information on reef status by relatively simple and inexpensive tools. We hope that this will encourage scientists and government officials to support the creation of an extensive worldwide network monitoring the health of reefs in real time, and including most remote and poorly explored reef areas.

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Demographic comparison of threatened Elkhorn coral, *Acropora palmata*, in the Caribbean: A case study in successful volunteer partnerships in a regional-scale monitoring program.

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Abstract. Due to severe and ongoing population declines, *Acropora palmata* received US federally threatened status in 2006. NOAA has since released a protocol for demographic monitoring of this genus in order to assess population status, and to encourage monitoring partnerships throughout the Caribbean (Williams et al 2006). In 2006, NOAA partnered with the SeaMester program to include monitoring sites along their sailing routes, including the British Virgin Islands (BVI), Bequia (St. Vincent Grenadines), and Green Island (Antigua). Monitoring data collected by the SeaMester program in the BVI are presented here, along with NOAA monitoring data from Curacao (Netherlands Antilles). Six fixed *A. palmata* monitoring plots were established in BVI, and 9 were established in Curacao. Within these plots, 12-15 randomly selected colonies have been measured annually and examined for signs of disease, predation, and other notable conditions. Live area index (LAI), and white syndromes, snail predation, and damselfish territory prevalence were statistically compared between the two regions. The relatively user-friendly protocol coupled with basic field trainings with partners allowed for substantial spatial expansion of this monitoring program. This broader monitoring coverage is needed to guide prioritized Caribbean-wide management based on observed population condition and resilience.

Key words: *Acropora*, demographic monitoring, *Coralliophila*, *Stegastes*.

Introduction

The elkhorn coral, *Acropora palmata*, is a spectacular shallow-water branching coral species that offers structural framework, protective habitat, and tourist-appeal to coral reef ecosystems. Like many other coral reef species, disease and bleaching events, hurricanes, and stressors associated with coastal development have driven dramatic population declines over the past 40 years (Gladfelter 1982, Nagelkerken and Nagelkerken 2004, Boulon et al 2005).

In response, *A. palmata* and congener, staghorn coral (*A. cervicornis*), were listed as threatened under the US Endangered Species Act (ESA) in May 2006 (NMFS 2006). To satisfy the ESA mandate for regular status updates, the National Oceanographic and Atmospheric Administration (NOAA) released a protocol for *Acropora* spp. demographic monitoring (Williams et al. 2006). The protocol aims to directly document population status, and to apply findings to management activities. Also, the protocol was designed to promote collaborative monitoring partnerships throughout the Caribbean.

Material and Methods

In January 2006, education staff from the SeaMester program volunteered to participate in monitoring efforts along its Caribbean sailing route, which provided a unique opportunity to address the significant gap in *A. palmata* monitoring and research in the eastern Caribbean. After receiving field training and guidance from the protocol authors, staff and students from the SeaMester program initiated *A. palmata* monitoring off Virgin Gorda and West Dog Island (British Virgin Islands), Bequia (St. Vincent Grenadines), and Green Island (Antigua). These complement existing NOAA monitoring locations in the Florida Keys, Puerto Rico, Navassa Island, and Curacao (Netherlands Antilles) (See Fig. 1). Results presented here will focus on monitoring efforts in Curacao and the BVI.

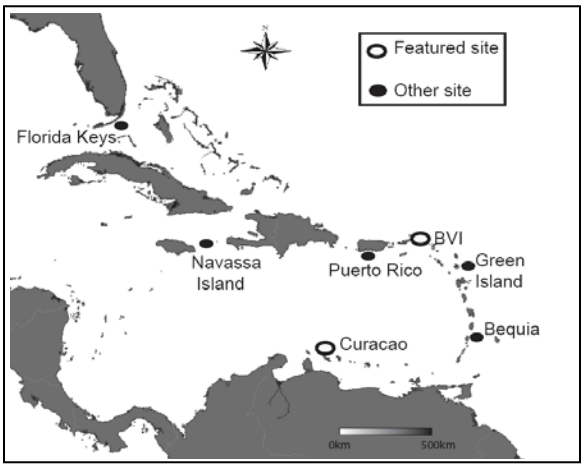

Figure 1: Current *A. palmata* demographic monitoring sites.

Detailed monitoring methods are available in Williams et al. (2006). In each monitoring region, we located 2-3 reef areas with moderately dense *A. palmata* colonies (avoiding thickets and isolated colonies). In these areas, we haphazardly established three 7m radius permanent monitoring plots, and within in each plot, 12-15 colonies were randomly selected for tagging and detailed monitoring. Each tagged colony has been annually measured, and examined for signs of disease, predation, bleaching, fragmentation, growth anomalies and other notable conditions.

Using size measurements, we calculated a live area index (LAI) for each tagged colony, where: LAI= $((\text{length} \times \text{width} \times \text{height})/3)^2 \times$ % live. This served as an index (not a direct estimate) of change in colony live tissue coverage. Importantly, LAI generally underestimates actual surface area, and this underestimate increases with colony complexity.

When additional resources were available, we also conducted annual total live tissue inventories (including recruitment) within each plot, genotype analysis, growth measurements, and exploratory regional mapping.

Datasets and photographs were received within one month of each monitoring event conducted by SeaMester staff and students. NOAA staff regularly addressed field questions, and datasets were quality-assured by verifying observations with colony photographs and through discussion with the observers.

We compared Curacao and BVI datasets for: a) white syndrome prevalence (including white-band disease (WBD), white pox (WPx), and rapid tissue loss (RTL)), b) average colony live area index (LAI), c) snail (*Coralliophila abbreviata*) predation prevalence (defined by regions of exposed, clean white skeleton along the colony live tissue edges), and d) three-spot (*Stegastes planifrons*) damselfish territory prevalence (defined by live tissue 'chimney' structures topped with algal tufts (Fig. 2).

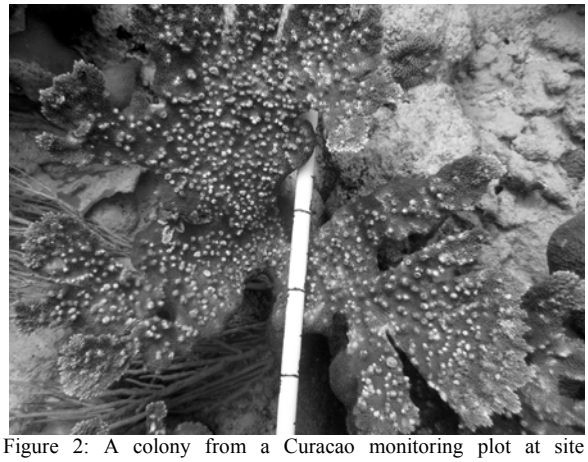
Figure 2: A colony from a Curacao monitoring plot at site 'SeaAqrm' impacted by an extensive three-spot damselfish (*S. planifrons*) territory. *S. planifrons* repeatedly bites areas of live coral tissue, which heal and overtime become chimney-like structures topped with fleshy macroalgae.

Datasets were first tested for normality and homogeneity of variance (Levene's test), and if needed, transformed ($\log_{10}$, square-root) to correct for failed assumptions. Usable datasets were statistically compared using a repeated measure (annual) hierarchical design ANOVA with survey plot nested within site and region.

Results

Live Area Index (LAI): Colony assemblages in the BVI and Curacao were very distinct, which was reflected in the live area index (LAI). A typical colony in the monitored areas of Curacao was relatively large, with many long branches, and low partial mortality (resulting in a high LAI). A typical colony in monitored areas of the BVI was small and mostly encrusting with sometimes a few short branches, and a moderate level of partial mortality (resulting in a low LAI). LAI varied significantly by region, and was twice as high for Curacao plots (Fig. 3). LAI increased slightly at all plots between 2006 and 2007.

Disease Prevalence: White syndrome (WPx, WBD, RTL) prevalence varied significantly by year and by region (Fig. 4). In 2007, white syndromes affected 23.9-56.9% of monitored colonies in Curacao, compared to 19.4-36.0% in 2006, and to 0-2.8% for the BVI in 2006. By 2008, white syndrome prevalence in Curacao fell to 14.6-29.3% of colonies affected, and healing lesions were frequently observed.

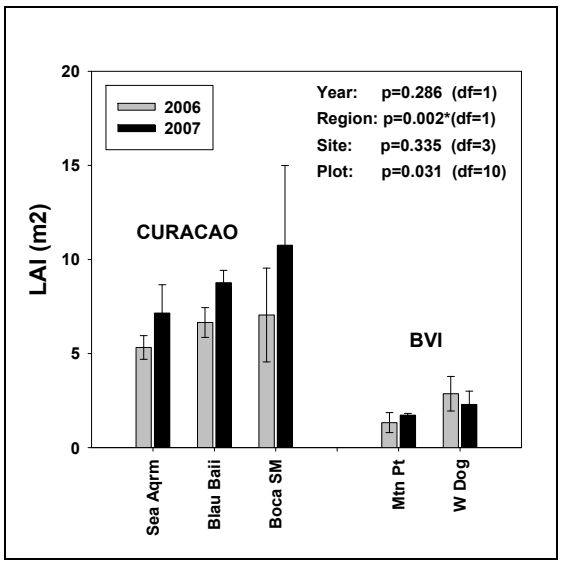

Figure 3: Mean colony live area index (LAI) (±1 SE) for *A. palmata* colonies in Curacao and the BVI in 2006 and 2007. *Indicates a statistically significant factor.

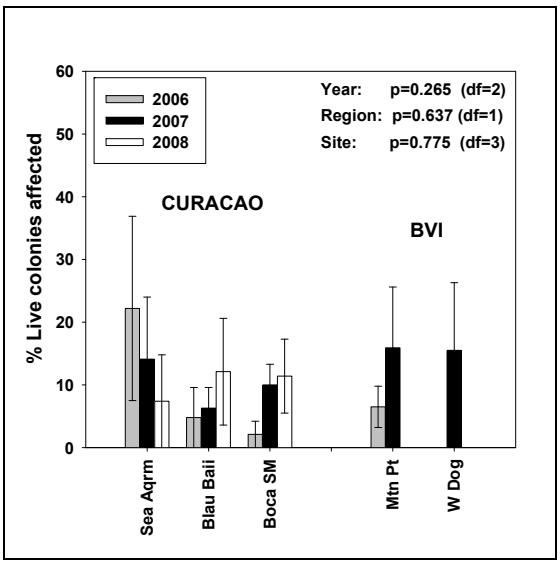

Figure 5: Mean prevalence (% live colonies affected) of snail (*C. abbreviata*) predation in Curacao in 2006-2008 and the BVI in 2006-2007 (±1 SE).

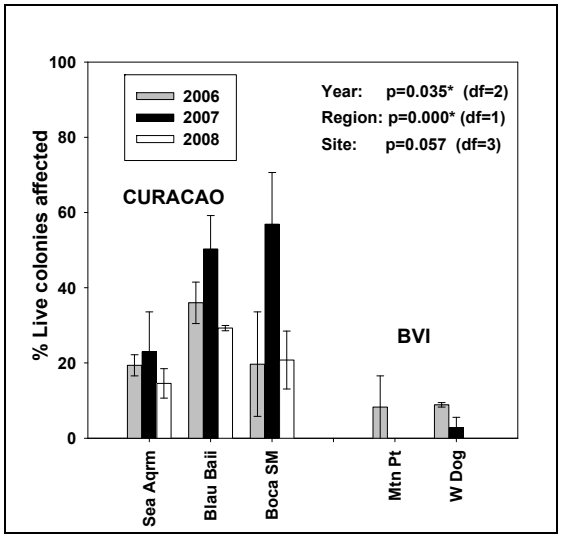

Figure 4: Mean prevalence of white syndromes (WBD, WPx, RTL) affecting *A. palmata* colonies in Curacao in 2006-2008 and the BVI in 2006-2007 (±1 SE). *Indicates a statistically significant factor.

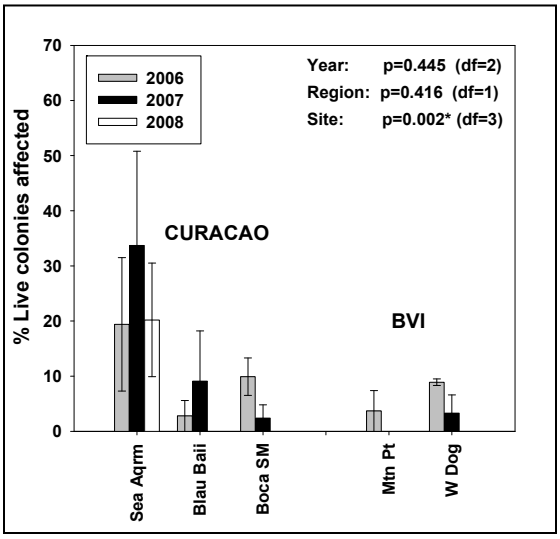

Figure 6: Mean percentage of *A. palmata* colonies impacted by three-spot damselfish (*S. planifrons*) territories in Curacao in 2006-2008 and the BVI in 2006-2007 (± 1 SE). *Indicates a statistically significant factor.

Snail Predation: The prevalence of live tissue grazing by the coral-eating snail, *C. abbreviata,* ranged from 2.1-22.2% of monitored colonies affected in Curacao, and 0-15.9% in the BVI. Predation prevalence was highly variable, and did not vary significantly by year, region or site (Fig. 5).

Damselfish Territories: Three-spot damselfish (*S. planifrons*) territories varied significantly by monitoring site (Fig. 6). Prevalence was generally low at all sites (ranging from 0-9.9% of monitored colonies affected, except Curacao site 'SeaAqrm,' which ranged from 19.4-33.7%) (Fig. 2, Fig. 6).

Discussion

Monitoring and Management: Regional differences in *A. palmata* populations, such as observed differences in 'reactions' to and recovery from threats, can have important implications for managers. Population variability based on the surrounding environment, habitat-driven morphology (Hubbard 1988), and genetics (Baums et al. 2005) allows certain populations to flourish under varying 'threat' circumstances. Regional monitoring programs such as this one can track population responses to threats, can assist managers in appropriate mitigation, and can

potentially help predict the likelihood of population survivorship, allowing managers to prioritize accordingly (Vardi et al. 2008).

Typical colony morphologies in Curacao and in the British Virgin Islands were very distinctive; an average colony in monitored areas of Curacao was relatively large and frondose, while a typical colony in BVI plots was small and mostly encrusting, sometimes with a few short branches. These morphology differences resulted in significant differences in LAI (Fig. 3), and can have important implications for asexual fecundity in this species (Fong and Lirman 1995, Williams et al. 2008). Thus, a typical colony in Curacao would be expected to yield greater asexual recruitment in a moderate storm or other fragmentation event, which suggests that the population is relatively better able to sustain itself reproductively. Importantly, LAI generally underestimates actual surface area (particularly for more complex colonies) (Williams et al. 2008), so the regional difference in actual live tissue was probably greater.

Monitored areas in Curacao recovered well following frequent White pox (WPx) observations. In 2007, white syndromes (usually WPx, but also sometimes WBD) affected 23.9-56.9% of monitored colonies in Curacao, compared to 19.4-36.0% in 2006, and to 0-2.8% for the BVI in 2006. By 2008, white syndrome prevalence in Curacao fell to 14.6-29.3% of colonies affected, evidence that colonies were able to overcome WPx, at least on the short-term. Partial mortality from WPx lesions in 2007 was often still visible on the affected colonies during 2008 surveys, but was usually characterized by an advancing line of live tissue working toward the center of the old lesion.

The long-term effects of three-spot damselfish territories are not well understood. In this study, territory prevalence was generally low at all monitored sites, ranging from 0-9.9% of colonies affected, except for Curacao site 'Sea Aqrm,' which hosted significantly more territories, ranging from 19.4-33.7% (Fig. 2, Fig. 6). Subsequent annual surveys will likely shed light on the long-term consequences of these territories on *A. palmata* colony condition.

Snail predation was low to moderate at the monitored sites (Fig. 5), ranging from 2.1-22.2% of monitored colonies affected in Curacao, and 0-15.9% of affected in the BVI. In areas with substantial *A. palmata* population declines, such as the Florida Keys, *C. abbreviata* grazing activities have been shown to be particularly detrimental, as snail density and predation increased on surviving colonies (Miller et al 2002). Observations of detrimental snail predation can potentially serve as justification for emergency removal by managers (Miller 2001). Snail predation will continue to be monitored at all sites.

Volunteer Involvement in a Monitoring Program: Threatened and endangered species receive a great deal of attention from the media, often creating public interest and volunteers willing to assist with fieldwork. This enthusiasm can potentially dramatically expand the spatial scale of regional monitoring studies. However, channeling this interest and participation into scientifically meaningful and consistent data can be challenging. For the *Acropora* spp. monitoring protocol (Williams et al. 2006), our successful partnership with the SeaMester program in the British Virgin Islands was facilitated by field trainings with experts during monitoring plot set-up and initial surveys. The SeaMester program was an ideal volunteer partner because of its on-board education staff with a background in marine biology, and because it allowed us to gather consistent data from remote areas that would otherwise be inaccessible.

Observations and data collected by SeaMester and other collaborators are always documented photographically, allowing NOAA staff to quality-assure data by reviewing a subset of observations (including all unusual observations). Any questions from the field crews were addressed as quickly as possible, and a consensus was obtained prior to final data entry.

The protocol (Williams et al. 2006) is designed with various levels of participation options, based on the time and expertise constraints of potential collaborators. Even if a potential partner is only able to collect very basic monitoring data, odd or alarming observations can serve as an 'early warning system' for further investigation by dedicated field teams.

Demographic *Acropora* spp. monitoring requires field training and advanced scuba diving abilities, particularly for *A. palmata* surveys (relative to surveys of *A. cervicornis*, which tends to inhabit deeper reef areas, and is slightly more compact and less structurally delicate). Monitoring sites are usually very shallow, and located along at the reef crest where wave energy and surge is maximized. Volunteers are required to have a certain degree of buoyancy expertise in order to conduct monitoring activities safely and without damaging corals.

Broad scale monitoring projects like this one offer a unique chance at understanding regional differences in *Acropora* spp. population response to a variety of challenges. As global climate change, disease outbreaks, and coastal development continue to affect the Caribbean region, our broader knowledge of *A. palmata* can potentially guide prioritized Caribbean-wide management based on observed regional population condition and resiliency.

Acknowledgements

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The Effects of Hurricane-Deposited Mud on Coral Communities in Florida

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Abstract. Widespread accumulation of mud on nearshore hardbottom was recorded during biological monitoring surveys for beach nourishment projects from Cape Canaveral to Miami along the Florida Atlantic coast and from Naples to St. Petersburg along the Florida Gulf of Mexico coast following the hurricanes of 2004. Permanent video transects were used for this study along with in-situ measurements of mud layer thickness. The surveys have indicated deposition of a relatively thin layer of fine sediments over extensive areas of hardbottom and thick accumulations at the base of sharp relief features. Storms suspend this fine material, resulting in turbid nearshore waters for long periods. The high adhesive capacity of the mud particles has enabled sediments to survive as a compact layer through the winter storms of 2004 through 2008. The effects have been lethal for many filter-feeding species while photosynthetic activity and recruitment opportunities are reduced for most benthic species. The 2007 monitoring data suggests significant mortality of octocoral recruits due to the smothering effects of mud, indicating that octocorals are not surviving beyond two to three years in this environment. It is hypothesized that the sediments were transported by hurricane-generated waves and currents from the deeper parts of the shelf.

Key Words: hurricanes, mud, recruitment, corals, octocorals

Introduction

Reefs and hardbottom communities with variable representation of scleractinian coral species and coral cover are widespread in the upper shelf of Florida. Between August 14 and September 26, 2004, four hurricanes (Charley, Frances, Ivan, and Jeanne) impacted the Florida shelf and coastline. Following these storms, widespread accumulation of mud on nearshore hardbottom and reefs was recorded from Cape Canaveral to Miami along the Atlantic coast and from Naples to St. Petersburg along the Gulf of Mexico (Gulf) coast. The nearshore distribution of mud is very wide with water depths ranging from 3 meters to at least 15 meters, and the offshore distribution ranges from approximately 50 to 60 meters to 1-2 kilometers from the shoreline. Very nearshore accumulation is characteristic of areas where vertical escarpments face the shoreline, such as Indian River County on the Atlantic side and Collier County on the Gulf side of Florida (Figure 1). Distant offshore accumulation was recorded in the Gulf of Mexico where the shelf is characterized by a very low gradient.

The mud has accumulated in a range of forms: relatively small pockets of a few decimeters in width in concave areas; attached to or perched on top of reefs and hardbottom ridges, subvertical to overhanging escarpments (ledges) and knolls; and a thin veneer of mud over sand, hardbottom and reefs. The thickness of accumulated mud varies from a few millimeters to over a meter. The content of the mud is variable with a visually higher carbonate component in south Florida in comparison to northern areas. For example, the mud in Indian River County is darker than the mud observed in Broward County.

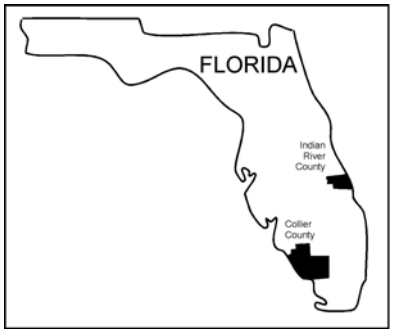

Figure 1. Location map of Florida counties mentioned in text.

The high adhesive capacity of the mud particles has enabled sediments to survive as a compact

layer through the winter storms of 2004 through 2008. Shallow nearshore locations are characterized by high wave energy. Storms suspend this fine material, resulting in turbid nearshore waters for long periods. Mud accumulations persist in these areas, both as a thin veneer over hardbottom and hardbottom communities and as thick accumulations (Fig. 2a). In some locations along the Indian River County shoreline, (central Florida Atlantic coast), accumulations of more than 20 cm in thickness were observed along the inshore edge of hardbottom at the base of scarps and ledges. The surveys have also indicated the deposition of a relatively thin layer of fine sediments over extensive areas of hardbottom. As a result, the visibility in nearshore waters has been dramatically reduced for several years. The summer of 2007 was the first opportunity of adequate water clarity for aerial photography to discern the shallow water hardbottom features in Indian River County.

Methods

Annual measurements of mud accumulation were performed from 2006 through 2008 on a nearshore artificial reef constructed as mitigation for impacts to nearshore hardbottom from a beach restoration project in Indian River County. Permanent transects were established for the evaluation of benthic community succession on the artificial reef. Monitoring methods involved permanent video transects and photo surveys with *in-situ* measurements of mud layer thickness to study the dynamics and effects of mud on benthic succession.

Two types of artificial reef were constructed as mitigation for impacts to nearshore hardbottom habitat: high-relief, high-complexity (HRHC) reef and low-relief, low-complexity (LRLC) reef. The high-relief, high-complexity artificial reef was designed to have a vertical relief of 0.9 to 1.8 m. Limestone boulders were partially stacked to achieve this vertical relief. The low-relief, low-complexity mitigation reef was designed to mimic a vertical relief of 0.3 to 0.6 m using a single layer of limestone boulders. The mitigation reef was constructed between May and September 2004; however, artificial reef placement could not be completed due to the passage of hurricanes Frances and Jeanne in September 2004. Therefore, a portion of the high-relief reef and the main portion of the low-relief reef were constructed in the summer of 2005. This phased construction enabled us to compare the influence of mud on benthic ecological succession.

Results

Accumulations of mud up to 30 cm in thickness were measured along the inshore edge and within spaces between boulders of the pre-hurricane artificial reef during the Year 1 baseline survey in August 2006. A thin cover of mud was also observed on the subhorizontal surfaces of the boulders. During the July 2007 survey, the mud persisted and was redistributed in a slightly thinner layer throughout the artificial reef, migrating into areas which had not been previously affected by mud. During the 2006 baseline survey, mud was not recorded on the post-hurricane high-relief reef; however, in July 2007, mud accumulations were recorded on this portion of the artificial reef. In July 2008, mud pockets increased to 15 cm in thickness along the portions of the post-hurricane reef constructed in 2005. Overall, mud accumulation is deeper along the western edge of the mitigation reef as compared to the eastern (i.e. seaward) edge.

The persistence of the mud layer on the pre-hurricane high-relief reef has led to the extensive development of cyanobacterial mats (Fig. 2b). The development of cyanobacterial mats is a very typical feature of mud accumulation throughout the study area. Mud-related cyanobacterial growth has had a dramatic smothering effect on octocoral recruits (*Leptogorgia* spp.) on the artificial reef. The total number of octocorals along the permanent transects decreased from 56 in 2006 to 16 in 2007. The surviving colonies suffered from partial coverage of cyanobacterial mats, and tissue loss and mortality were observed under epiphytic overgrowth (Fig 2c). A similar effect was observed in southeast Florida; however, a small number of *L. virgulata* colonies survived for several years despite partial burial by mud.

In 2006 and 2007, the dominant functional group on the post-hurricane artificial reef was fleshy macroalgae. Overall percent cover of macroalgae at the post-hurricane high-relief reef in July 2007 was 77.6% compared to an overall average of 5.3% at the pre-hurricane high-relief reef (Fig 3). The macroalgal community on the pre-hurricane reef was initially dominated by calcareous algae due to its ability to survive under a veneer of sediments. A turf algae/cyanobacteria matrix replaced calcareous algae as the dominant functional groups on the pre-hurricane artificial reef in July 2007.

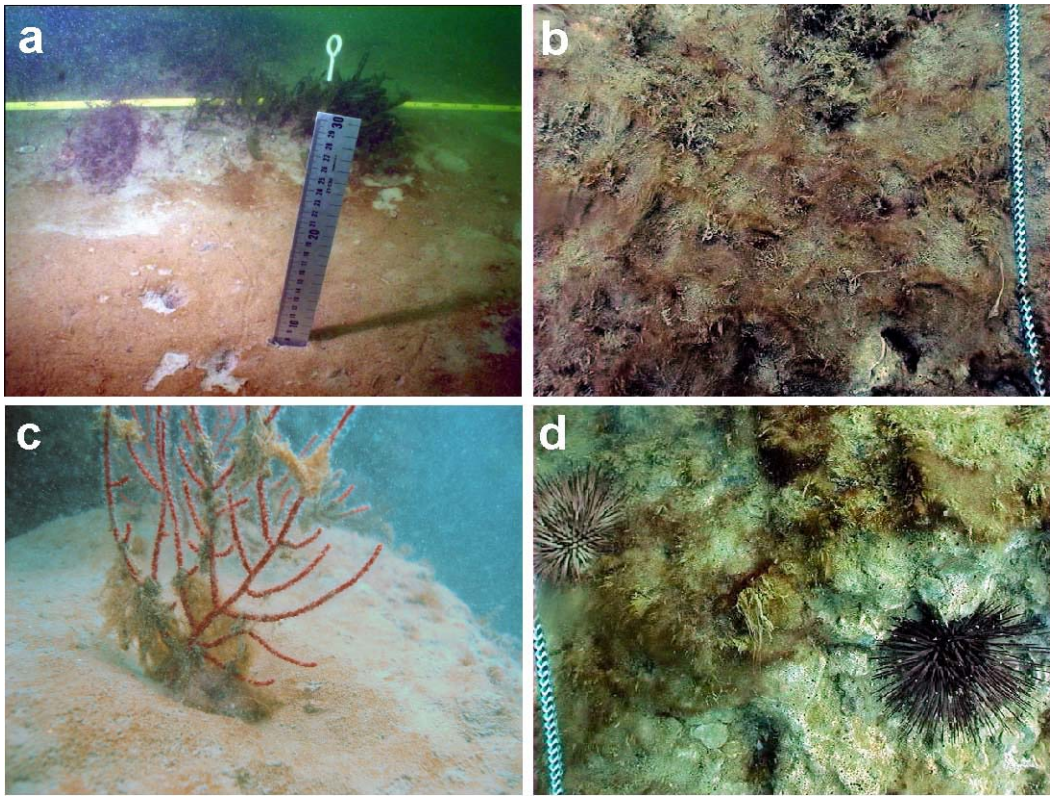

Figure 2. a) Mud layer of 8-10 cm covers extensive areas of hardbottom at depth of 5 m, offshore of Sarasota County, FL; b) Cyanobacterial mat over a thin layer of mud covering both substrate and algae on the pre-hurricane artificial reef, Indian River County, FL; c) Accumulation of mud flakes on octocoral, *Leptogorgia hebes,* stimulate growth of cyanobacteria and algae which smother octocoral tissue; d) Sea urchins scrape mud and cyanobacteria, exposing clean limestone surface for recruitment opportunities.

The 2007 survey revealed a three-fold increase in the sea urchin population on the artificial reef in comparison to 2006 (118 versus 37). The number of urchins (*Arbacia punctulata* and *Echinometra lucunter*) along the six permanent transects increased to 296 in 2008.

The increase in the urchin population is positively correlated with the increase in coral recruitment between 2006 and 2008. Ten small coral colonies (<2 cm in diameter) were recorded during the 2007 survey compared to two colonies in 2006. Twenty-one *Oculina* sp. colonies were counted along the six video transects during the 2008 survey. *In situ* counts along one of the 20-meter monitoring transects on the pre-hurricane reef documented 32 *Oculina* sp. recruits <2 cm in diameter during the July 2008 survey.

Discussion

The 2006 and 2007 annual surveys have demonstrated that mud deposition has had a greater effect on benthic succession and community composition than the difference in vertical relief in the Indian River County artificial reef. The continual resuspension and deposition of mud on the benthic community on the reef constructed in 2004 (pre-hurricane) has resulted in recurrent disturbance events which limit macroalgal colonization and growth.

Scleractinian corals are a minor component of the nearshore hardbottom community in Indian River County and are generally represented by only three species, *Siderastrea siderea*, *Oculina varicosa* and *Phyllangia americana*. Recruitment and survival of small colonies has been inhibited by mud deposition on both the natural nearshore hardbottom and artificial reef in Indian River County. However, the proliferation of sea urchins on the artificial reef between 2006and 2008 has increased recruitment opportunities. Sea urchins remove the mud and cyanobacterial mats while grazing, exposing clean limestone surface available for coral recruitment (Fig. 2d). The high density of *Oculina* recruits in the 2008 survey was observed in areas of bare substrate scraped clean of mud by urchin activity. In comparison, no coral recruits were observed along this transect during the 2007 survey.

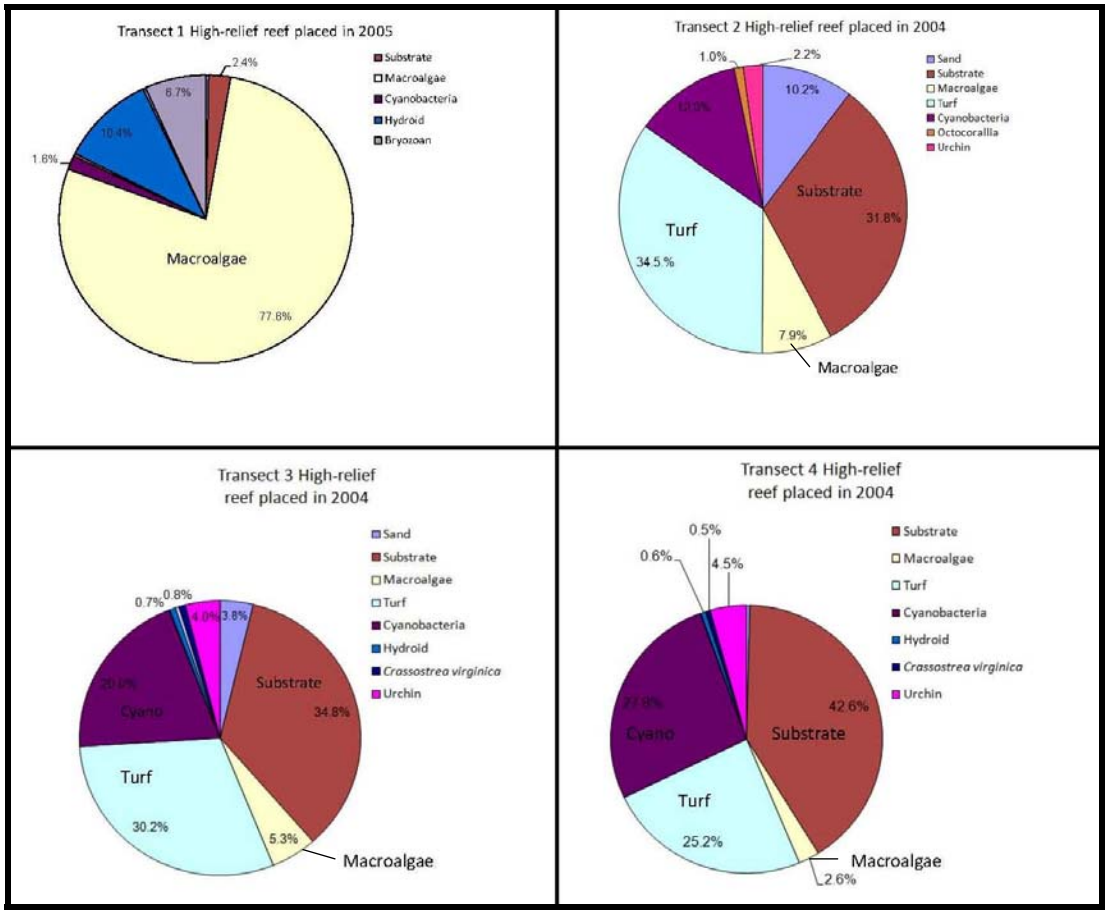

Figure 3. Percent cover of the major functional groups on the Indian River County artificial reef. Note dominance of macroalgae on the post-hurricane reef versus substrate and cyanobacteria on the pre-hurricane reef. Survey date is July 2007.

Scleractinian corals are a considerably larger component of hardbottom communities in southwest Florida; however, scleractinian coral diversity is low. The most common species are *Siderastrea siderea*, *Solenastrea bournoni* and *S. hyades*, *Cladocora arbuscula* and *Phyllangia americana*. The impact of mud on corals varies considerably for the same species with colony size, shape, location in nannorelief features and depth of mud layer.

Siderastrea siderea and *Solenastrea* spp. are notorious for their tolerance to turbid water and sedimentation. However, this mud event has resulted in an overwhelming accumulation of fine material which has remained within hardbottom areas for several years. Larger, vertically-developed colonies exhibit higher survivorship than encrusting and flat colonies, which can undergo fission and partial mortality. Corals are actively removing mud from their surface, and mud particles are continually resuspended by wave and current action. Continuous active sediment removal slows growth rates since energy is diverted to mucus production and particle removal rather than feeding and photosynthesis/ symbiosis, potentially resulting in coral death (Riegl and Branch, 1995).

Cladocora arbuscula forms small colonies less than 10 cm in diameter. *C. arbuscula* colonies are typically less than 5 cm high in Collier County. Several colonies of *C. arbuscula* were observed buried and dead while some were partially buried and dead. The cryptic species, *Phyllangia americana*, settles and grows mostly on subvertical and overhanging features, avoiding sedimentation impacts associated with horizontal surfaces. During our three years of post-hurricane surveys in Collier County, the only coral species observed recruiting within the areas of nearshore mud accumulation was *P. americana*.

The effects of mud deposition have been lethal for many other filter-feeders while photosynthetic activity and recruitment opportunities have been reduced for most benthic species. Small sponges (for example, *Cinachyra alloclada,*), zoanthids, Actiniaria, tunicates, etc. were observed dead under mud cover throughout the study area on both the Gulf and Atlantic sides of Florida. For example, 30 tunicates were recorded on the pre-hurricane high-relief artificial reef in Indian River County in 2006, while in 2007, only 5 tunicates were recorded along

the same permanent transects. Under normal conditions, exponential growth of cover by tunicates during the first few years of succession is a characteristic feature of artificial reefs in South Florida.

Conclusions

It is hypothesized that the mud was transported by hurricane-generated waves and currents from the deeper parts of the shelf, where this material normally accumulates. We cannot suggest any other source for such an enormous amount of mud to be transported during such short-term events such as hurricanes. Large accumulations of mud were observed in southeast Florida in 1960 after Hurricane Donna at depths of 25 to 45 meters. These accumulations were later removed by currents and waves (E. Shinn, personal communication). Shinn et al. also described the deposition of mud layers over ooid sands in the very shallow realm of banks south of Joulters Keys and Cat Key in the Great Bahama Bank after Hurricane Andrew in August 1992 (Shinn et al., 1993).

Our observations of mud in Collier County and Palm Beach County are similar to the descriptions of interlacing mud with coarse post-hurricane sand described by Shinn et al. (1993). It is possible that the impacts of mud on reefs and hardbottom communities are more widespread than suggested by our observations and surveys. It is not possible to evaluate the extent and consequences of this impact on benthic communities and individual groups of organisms without considerable additional study. However, it is clear that the effects are long-lasting.

We have observed that mud masses are able to move along or around features; however, we are uncertain if the sediments are moving as liquid, gel-like body or are resuspended and accumulate in adjacent areas in the leeward side of relief features. The latter appears to be more plausible; however, the sediment dynamics have not been studied. The redistribution and accumulation of mud in the areas of the Indian River County artificial reef constructed after the hurricanes of 2004, which were previously unaffected by mud deposition, are clear evidence that the mud masses are mobile in the nearshore environment.

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Population status of *Acropora* corals in the Florida Keys

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Abstract. Population declines of staghorn coral (*Acropora cervicornis*) and elkhorn coral (*A. palmata*) are often-cited examples of Caribbean reef change since the 1970s, due, in part, to disease and localized effects from storms and predation. Both corals were listed as threatened on the U.S. Endangered Species List based upon range-wide decline and poor recovery. A spatially intensive survey undertaken in the Florida Keys of *Acropora* corals quantified habitat distribution, colony abundance, size, and condition at 235 sites spanning over 200 km in 2007. A two-stage stratified sampling design using belt transects incorporated cross-shelf habitats and no-fishing management zones from < 1 m to 15 m depth. *A. cervicornis* was widely distributed among sites and habitats and was particularly abundant on patch reefs, with up to 1.22 colonies/m^2 and surface area coverage of 2%. *A. palmata* was abundant on shallow spur and groove reefs, with up to 1.25 colonies/m^2 and surface area coverage of 25%. Although the prevalence of disease is relatively low, both species continue to suffer predation, as well as physical impacts from lost fishing gear. Predicting the future of these corals in Florida requires information about both their present-day ecology and geologic history in Florida.

Key words: *Acropora*, benthic, coral, marine protected area, population, stratified sampling.

Introduction

The declines in abundance of two of the principal Caribbean reef-building corals, staghorn coral (*Acropora cervicornis*) and elkhorn coral (*A. palmata*), are often-cited examples of the changes in western Atlantic reefs that have occurred during the past several decades (Bruckner 2002; Gardner et al. 2003). The causes of these declines, which began in the late 1970s, include large-scale factors such as coral bleaching and disease, especially white band disease (Gladfelter 1982), as well as smaller-scale effects from storms and predation by corallivorous snails and damselfishes (Miller et al. 2002). Both coral species were under consideration for addition to the U.S. Endangered Species List since the early 1990s and were determined to be "threatened" based upon range-wide population declines and poor recovery (*Acropora* Biological Review Team 2005).

Although there is increased awareness of the fragility of Atlantic *Acropora* corals to further potential population decline, there is surprisingly little information on density structure, size, and population abundance for wider Caribbean reef areas. Notable exceptions to this pattern include recent population assessments of *A. palmata* in the U.S. Virgin Islands, southern Caribbean, and in the Florida Keys at one reef (Miller et al. 2002; Mayor et al. 2006; Zubillaga et al. 2008). While some recovery is apparent in localized areas, populations of both species remain depressed well-below historical levels, including the Florida Keys (Dustan and Halas 1987; Porter and Meier 1992), and threats continue that will potentially inhibit population recovery (*Acropora* Biological Review Team 2005).

To ascertain the current population status of both *Acropora* species, we conducted an intensive assessment of the spatial distribution, colony abundance, size, and condition of both species throughout the Florida Keys, including a large area of the Florida Keys National Marine Sanctuary (FKNMS) and Biscayne National Park (BNP) during 2007. The surveys were an outgrowth of previous efforts conducted by the authors dating back to 1999 to quantify the abundance and condition of coral reef benthos throughout the FKNMS. Data obtained from these earlier efforts, together with existing habitat mapping information for the FKNMS, were used to guide the sampling of *Acropora* corals along ~200 km of the Florida Reef Tract. The goals of the survey were to determine patterns in habitat distribution, coral colony density, colony size, condition, and total population abundance estimates (Miller et al. 2007).

Material and Methods

During June-August 2007, surveys at 235 sites were completed along ~200 km of the reef tract from northern Biscayne National Park to SW of Key West (Fig. 1). Previous surveys dating back to 1999 aided in optimizing a sampling plan for obtaining abundance and size distribution estimates for the two *Acropora* corals. A two-stage stratified random sampling design incorporated nine unique habitat

types (Table 1), as well as areas inside and outside of FKNMS no-take zones. The statistical design features are detailed in Smith et al. (in press).

To control for spatial variation in population abundance metrics, we divided the Florida Keys survey domain into strata based upon: 1) habitat class; 2) geographic region; and 3) management zones of the Florida Keys National Marine Sanctuary (FKNMS) and Biscayne National Park (BNP). Cross-shelf habitats were designated using regional benthic habitat maps (FDEP 1998). The habitat classification scheme accounted for features that correlate with benthic fauna distributions, including cross-shelf position, topographic complexity, and the proportion of sand interspersed among hard-bottom structures. A geographic regional stratification variable was used to account for oceanographic and geological features in the Florida Keys that influence the distribution, community dynamics, and biotic composition of reefs (Marszalek et al. 1977; Shinn et al. 1989). FKNMS no-take zones were incorporated as a third stratification variable that delineated areas open and closed to consumptive activities.

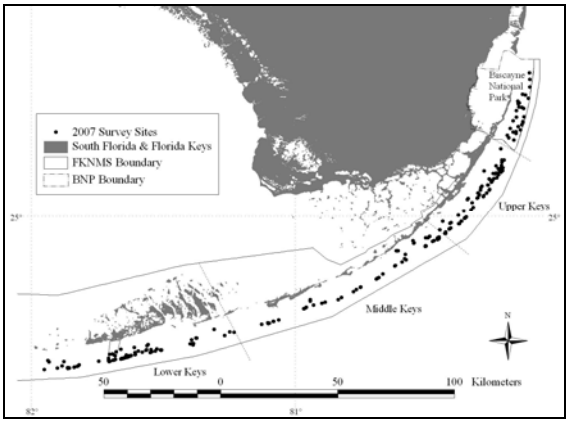

Figure 1: *Acropora* coral sampling locations in the Florida Keys.

A geographic information system (GIS) containing digital layers for benthic habitat (FDEP 1998), bathymetry, and no-take marine reserve boundaries was used to facilitate delineation of the sampling survey domain, strata, and sample units. Map resolution was such that the survey domain was divided into a grid with individual cells of size 200 m by 200 m (40,000 m^2) that defined unique habitat classes (Table 1). A two-stage sampling scheme following Cochran (1977) was employed to control for spatial variation in population metrics at scales smaller than the grid cell minimum mapping unit. Grid cells containing targeted reef and hard-bottom habitats were designated as primary sample units. A second-stage sample unit was defined as a belt transect of fixed area (15-m x 1-m in dimension) within a primary sample unit. The size of an individual primary sampling unit allowed divers to swim to the location of any given second-stage sampling unit from a moored or anchored vessel.

Table 1: Characteristics of study sites in the Florida Keys sampling domain. Available sites reflect the number of 200 m x 200 m cells containing particular habitat types based upon FDEP (1998)

Habitat	Depth (m)	Sites (% effort)	Sites available
Mid-channel patch reef (MPR)	0.9-2.7	36 (15.3)	3,532
Offshore patch reef (OPR)	2.1-14.6	42 (17.9)	1,170
Hard-bottom matrix	2.7-5.8	4 (1.7)	79
Shallow hard-bottom (LHBS)	2.7-7.0	25 (10.6)	972
Inner line spur and groove (IRT)	1.5-6.1	8 (3.4)	87
High-relief spur and groove (HSG)	0.6-9.4	51 (21.7)	238
Deeper hard-bottom (LHBD)	6.7-13.7	15 (6.4)	1,962
Patchy hard-bottom (PHBD)	4.6-11.3	21 (8.9)	956
Low-relief spur and groove (LSG)	7.6-16.2	33 (14.0)	2,825
Sampling Design Total	0.6-14.6	235 (100)	11,821

The underwater surveys consisted first of locating randomly selected, pre-determined coordinates with a differential global positioning system. The original sampling list included 180 sampling locations, with an additional 145 alternate sites. If the original waypoint was not the intended habitat, we sampled the closest alternate site. Once on-site, a two-person benthic diver team oriented four transect tapes 15 in length, marked in 1-m increments, along the bottom, and surveyed an area 0.5-m out from each transect side. Transects were placed in a haphazard fashion, but in a way that adequately represented the habitat at the randomly selected site coordinates. Once transects were deployed, divers determined the depth range along the transect using a digital depth gauge, as well as the maximum vertical relief using a 50-cm scale bar. Any *Acropora* corals that were observed within the 15-m x 1-m belt transects were counted, measured, and assessed for colony condition. For this study, a colony was defined as a patch of continuous live tissue (ramet). In cases where a skeletal unit, possibly representing a single genet, was divided into one or more patches of tissue with clearly defined boundaries, each patch was considered a separate ramet. Measured dimensions of ramets were used to estimate colony surface area using applicable surface area formulas. The condition measurements included an assessment of bleaching, disease, predation, and overgrowth by algae, sponges, and other biota.

Statistical estimation procedures for population abundance metrics (proportional transect frequency, density, total abundance) for a two-stage stratified random sampling design were adapted from Cochran (1977), and computations were carried out using SAS statistical software. Domain-wide mean and variance estimates of density were obtained from weighted averages of strata means and variances. A stratum weighting factor was the proportion of the stratum area relative to the overall survey domain (see Table 1). Similar procedures were used to estimate proportions such as frequency of transect occurrence. Stratum abundance (absolute number of colonies) was estimated by multiplying stratum density by stratum area. The same principle was used to estimate the variance of stratum abundance. Domain-wide abundance and associated variance were obtained by summing the respective strata estimates over the entire survey domain. Design estimation of means, proportions, totals, and their associated variances does not depend on the probability distribution of the underlying observations (Cochran 1977). As such, statistical testing for differences is done by constructing confidence intervals directly from standard errors of either a stratum-specific or domain-wide metric. Statistical comparisons of means were conducted by calculating confidence intervals (CI) based upon the equation CI = mean ± t[α, df] * SE (standard error), with SE estimated by the two-stage stratified sampling design (Cochran 1977). Confidence intervals were adjusted for multiple comparisons using the Bonferroni procedure. While this adjustment made for relatively conservative statistical testing, it reduced the probability of spurious significant pair-wise comparisons. The experiment-wise error rate was held at α = 0.05 and the comparison-wise error rate was adjusted based on the number of multiple comparisons (comparison-wise error rate = α /c, where c = k (k-1)/2). Colony abundance estimates structured by habitat and by colony surface area size were computed using the two-stage design (Cochran 1977).

Results

Staghorn coral (*Acropora cervicornis*) was observed in the general survey area at 55 of the 235 sites (23%) and was recorded within belt transect boundaries at 45 sites (19%). The habitat distribution of *A. cervicornis* was broader than *A. palmata*, with colonies found in all but one of the nine habitats (Fig. 2). *A. cervicornis* was frequently encountered on mid-channel and offshore patch reefs, as well as inner line reef tract sites, and by comparison was infrequently encountered on the deeper fore reef. Statistical comparisons of proportional transect frequency yielded a significance difference (P < 0.002, Bonferroni-adjusted α) between offshore patch reefs and low-relief spur and groove. A total of 508 *A. cervicornis* ramets were counted and mean (± 1 SE) colony density (no. ramets per m^2) ranged from 0.094 ± 0.030 on offshore patch reefs to ≤ 0.01 in four of the lower-relief fore reef habitats (Fig. 2). The greatest mean (± 1 SE) site level densities (0.683-1.217) occurred on mid-channel and offshore patch reefs. Despite this variation, no significant differences (P > 0.002, Bonferroni-adjusted α) in mean colony density among habitats were detected. Abundance estimates indicate that there are perhaps ~13.8 ± 12.0 million *A. cervicornis* colonies in the sampling domain, with nearly 90% on mid-channel and offshore patch reefs (Table 2).

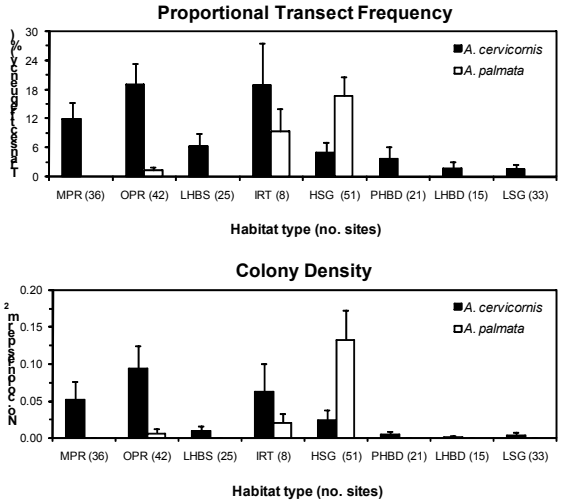

Figure 2: Mean proportional transect frequency (% of transects present) and colony density (no. colonies per m^2) for *Acropora cervicornis* and *A. palmata* in the Florida Keys. Error bars are +1 SE and numbers in parentheses on the x-axis are the number of sites sampled in each habitat. See Table 1 for habitat abbreviations.

Most *Acropora cervicornis* colonies were relatively small (< 0.5 m in max. diameter), although there were some mid-channel and offshore patch reefs with larger thickets. The percentage of live tissue surface area per m^2 of substratum was greatest on several mid-channel and offshore patch reefs, with upwards of 2% cover at individual sites. Mean ± 1 SE percent cover by habitat type was < 0.2% for all habitats, with offshore patch reefs (0.16 ± 0.06%), inner line reef tract (0.11 ± 0.06%), and mid-channel patch reefs (0.10 ± 0.05%) yielding the greatest habitat-level cover, albeit at very low values. Population abundance estimates structured by ramet surface area indicate that ~67% of the *A. cervicornis* colonies in the Florida Keys are less than 150 cm^2 in surface area (Fig. 3). Of the colonies assessed for condition, there were no obvious signs of white band disease, white plague, tissue necrosis, or *Coralliophila* predation.

Nine colonies (2.2%) had obvious signs of damselfish predation. Entanglement with lobster trap rope was common, especially on patch reefs, and of the 78 patch reefs sampled, more than 90% of sites, including Sanctuary no-take zones, contained remnant lobster trap debris. There were several instances where *A. cervicornis* colonies were entangled and obvious tissue damage and colony breakage resulted.

Table 2: Population abundance estimates (95% CI) for *Acropora cervicornis* and *A. palmata* in the Florida Keys sampling domain partitioned by habitat type. See Table 1 for habitat abbreviations

Habitat	*A. cervicornis*	*A. palmata*
MPR	7,391,961 (6,586,650)	0 (0)
OPR	4,656,900 (2,955,347)	295,989 (545,865)
LHBS	388,849 (406,738)	0 (0)
IRT	217,527 (270,435)	72,509 (80,934)
HSG	224,028 (269,877)	1,266,381 (744,035)
PHBD	237,554 (344,767)	0 (0)
LHBD	106,458 (213,021)	0 (0)
LSG	550,372 (958,157)	0 (0)
Total	13,773,647 (12,004,991)	1,634,879 (1,370,835)

Elkhorn coral (*Acropora palmata*) was observed in the general survey area at 24 of the 235 sites (10.2%) and was recorded within belt transect boundaries at 19 sites (8.1%). The habitat distribution of *A. palmata* was much narrower than its congener, with colonies found along belt transects in three of the nine habitats (Fig. 2). *A. palmata* was most frequent on high-relief spur and groove reefs and statistical comparisons of proportional transect frequency illustrated a significance difference between this habitat and five of the other habitats surveyed ($P < 0.002$, Bonferroni-adjusted α). A total of 403 *A. palmata* ramets were counted and mean (± 1 SE) colony density (no. ramets per m^2) ranged from 0.133 ± 0.039 on high-relief spur and groove to zero in five habitats (Fig. 2). The greatest mean (± 1 SE) site-level densities (0.833-1.250) all occurred in high relief spur and groove; this habitat type yielded a significantly greater mean colony density than five of the other seven habitats ($P < 0.002$, Bonferroni-adjusted α). Abundance estimates indicate that there are perhaps ~1.6 ± 1.4 million *A. palmata* colonies in the sampling domain, with nearly over 80% occurring on high-relief spur and groove reefs (Table 2).

Acropora palmata colony sizes showed a significantly greater range compared to its congener, and we were encouraged to find several sites with relatively large (> 0.5 cm diameter) colonies. High-relief spur and groove reefs yielded the largest colonies and percent cover values. Although mean percent cover on the 51 high-relief spur groove reefs sampled was 1.6 ± 0.6%, site-level cover was greater than 8% at several reefs and ranged up to 25%. These sites yielded the largest colony sizes, with several sites yielding mean surface areas of > 1,000 cm^2 per colony. Population abundance estimates structured by ramet surface area indicate that although ~50% of the *A. palmata* colonies in the Florida Keys are less than 250 cm^2 in surface area, many larger colonies still remain (Fig. 3). Of the *A. palmata* assessed for condition, ~5% were affected by predation by *Coralliophila* snails and damselfishes. We were discouraged to find lobster trap rope entangled in thickets of live colonies, including some within Sanctuary no-take zones, but were encouraged by the absence of visible diseases such as white band and white pox.

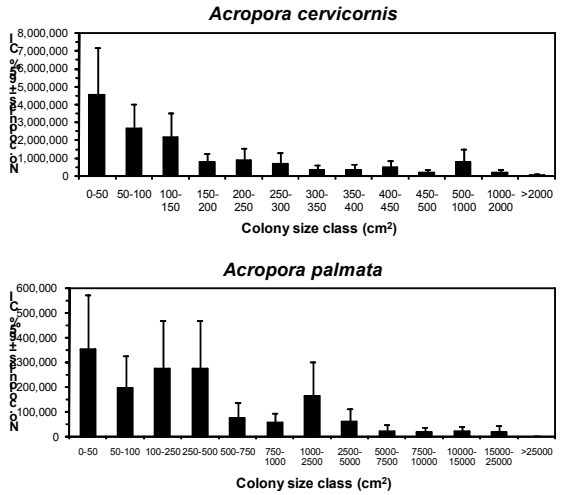

Figure 3: Population abundance estimates by ramet surface area for Florida Keys *Acropora cervicornis* and *A. palmata*. Error bars are 95% confidence intervals. Note the abundance scale change between the two species.

Discussion

This effort is one of the few studies to conduct population estimates of any coral species among a diversity of habitats representing a range in cross-shelf position and depth. For western Atlantic *Acropora* corals in particular, we are aware of only a handful of studies that attempted to derive total colony abundance estimates structured by habitat type and/or colony size (e.g. Miller et al. 2002; Mayor et al. 2006; Zubillaga et al. 2008). Population census results from a large area of the south Florida shelf indicate that both *Acropora* species are aggregated in particular habitat types, especially on the outer platform margin, in habitats noted for historically significant thickets of colonies. However, site-level densities were well below 1 colony/m^2 for both species at most sites sampled, and it is clear that the abundances of these corals are currently far below historical reports in the study area (Dustan and Halas 1987; Porter and Meier 1992). These results are similar to other studies in the region (Bruckner 2002; *Acropora* Biological Review Team 2005).

Acropora colony size distributions were skewed towards mostly smaller (< 100 cm^2) colonies, although larger thickets, especially *A. palmata*, were still present at some locations, especially in high-relief spur and groove habitats. Disease prevalence and evidence of predation from damselfishes and gastropods were low (« 1% of all colonies). We were encouraged to find relatively extensive thickets of *A. palmata* at several bank reefs scattered throughout the Florida Keys, and most of these reefs are currently zoned as no-take marine reserves. However, physical damage from derelict fishing gear, especially trap debris, poses what we believe to be a significant and ongoing impact to extant colonies, even within Sanctuary no-fishing zones.

Population abundance estimates for the Florida Keys illustrate considerable spatial variability, but nonetheless indicate that there are perhaps millions of extant colonies of each species in the study area, not including thickets of *A. cervicornis* to the north of the reef tract offshore of Ft. Lauderdale. At the same time, genetic diversity may be relatively low for both corals and is clearly a research need. Coupled with life history factors, lower genetic diversity may render both corals susceptible to ongoing impacts from storms, disease, and predation, suggesting that current conditions are perhaps not conducive to the recovery of both corals to 1960s or 1970s "baseline" levels (Williams et al. 2008).

What is apparent from these data is that the distribution and abundance patterns of the two species are clearly different, perhaps necessitating different management approaches. Although 34 different spur and groove reefs, including inner line reef tract, were sampled, our results indicate that significant *A. palmata* stands remain at only a handful of sites. While most of these sites are within existing FKNMS no-take zones, predation by snails and damselfishes, as well as physical impacts from lost fishing gear, is prevalent. The distribution pattern of *A. cervicornis* reflects the importance of patch reefs to the possible recovery of this species, which contrasts with historically abundant stands on the deeper fore reef. While there are over 5,000 patch reef sites on the south Florida shelf, *A. cervicornis* is very patchily distributed, and the factors responsible for this pattern are not well known. Promising management options for the recovery of *Acropora* corals have not been well defined, yet there are obvious actions that can be taken at the local level to enhance survival of existing populations that include removing fishing debris and minimizing the potential for further impacts to reefs.

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Observations of a Red Sea Fringing Coral Reef under Extreme Environmental Conditions

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Abstract. A four-year study collected the first comprehensive time-series data from a small Red Sea fringing reef located at the extreme northernmost latitude of tropical coral reefs. Corals here are exposed to 2-6° C daily water temperature changes with seasonal variations exceeding 14-16° C, and salinities between 43 and 45 ppt. Annual reef surveys included random photo quadrats, fixed video transects, and environmental measurements. In just four years, this pristine reef ecosystem was subjected to nearby shipping-port construction, rapid coastal urbanization, a bleaching event, and an oil spill. Results indicate that of the approximately 35 coral taxa known to survive here, just six compose 94% of those on this reef. Between 2004 and 2007, statistically significant changes were found in reef health indicators including a 50% increase in dead coral, 58% increase in sea urchins, and decreases in biodiversity and fish abundance. Despite all this, corals here appear to be well adapted to extreme environmental conditions. This ongoing research establishes a reference point for coral reef studies in this region and will be key to future local conservation efforts.

Key words: Red Sea, fringing reef, Gulf of Suez, marginal reefs, oil spill, bleaching, salinity extremes.

Introduction

The impact and rate of change of manmade influences on pristine fringing coral reefs, as well as the organisms that inhabit them, are not well documented. Fringing reefs grow close to shore in relatively shallow water and few fringing reefs remain in good health due to their accessibility and proximity to pollution sources. Although Red Sea reefs are cited among the most diverse in the world (Loya 1972), Red Sea corals are not well studied (Rinkevich 2005). Oceanographic and biologic data and long-term coral reef studies in the Gulf of Suez are nearly non-existent.

The reef investigated (dubbed "Zaki's Reef" after the first author's grandfather) is located in the Red Sea's Gulf of Suez near the most northern latitude for subtropical coral reefs. The very shallow (50 - 80 m deep) Gulf of Suez lies adjacent to an extremely arid desert where rainfall is minimal, evaporation rates are high, and freshwater inputs are non-existent. The persistent trade winds and extreme temperatures result in high salinity (43-45‰) and large daily and seasonal temperature fluctuations. High temperatures and salinity reduce oxygen solubility, which can stress many species of reef-dwelling organisms. The organisms that thrive here must be able to tolerate these environmental extremes. A recent study (Moustafa 2000) estimated that 335 species of corals are found in the Red Sea, yet only 35 species have been identified in the Gulf of Suez.

There are no known long-term studies investigating fringing reefs in the study area prior to the research reported here. Therefore, the main goal for this study was to establish a knowledge base for fringing coral reefs in the Gulf of Suez and provide basic information for future reef management, as reef-related activities generate a significant portion of Egypt's tourism revenue.

The effects of natural and manmade influences on a pristine, isolated fringing coral reef located at an extreme northern latitude were documented annually for four summers. Pollution from shipping and oil spillage pose a significant threat as does rapidly expanding coastal urbanization and other anthropogenic influences new to the region. Specific objectives were to: 1) assess reef condition and identify dominant coral taxa; 2) identify local natural and manmade stressors, 3) assess the reef's resilience to recent natural and manmade influences; 4) document changes in indicators of reef health including incidence of disease; 5) conduct annual fish surveys; 6) evaluate reef sediment components; 7) assess water quality, and 8) share results since data on this region are nearly nonexistent.

Material and Methods

Zaki's Reef is a small fringing reef (approx. 0.8 km x 0.1 km) located in Gulf of Suez (29° 32' N and 32° 24' E). The reef averages only one to two meters in depth at mean low tide, and can be accessed by a short swim

from the beach (Fig. 1). Methodologies used to sample this reef followed universally accepted protocols (Hill and Wilkinson 2004). Underwater videography was repeated annually (Page et al. 2001) to document changes along five 10m transects located across the reef (Fig. 2). Fish surveys were conducted by visually logging and digitally recording taxa and their abundance during five-minute observation periods at three of the 10m transects.

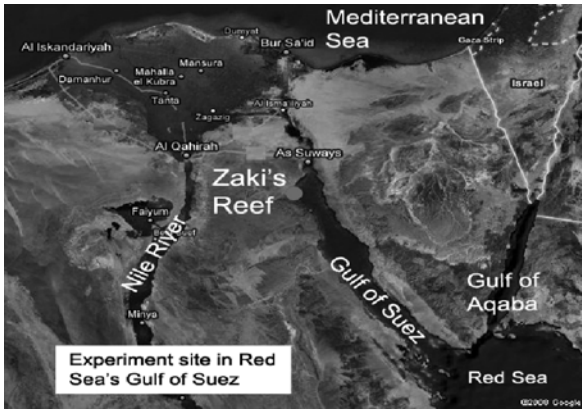

Figure 1: Location of Zaki's Reef in the Red Seas Gulf of Suez, approximate 80 km south of the highly trafficked Suez Canal.

A reef-wide random quadrat (0.5 m^2) photo survey captured bottom substrate from across the reef to establish spatial variation, percent coral cover, diversity, and dominance. Quadrat survey swim direction and distance were randomly determined from the roll of two dice. Each year, statistics for percent bottom coverage by coral taxa were derived from the analysis of approximately 120 randomly collected quadrat photos.

Accessory measurements included salinity and pH measured across the reef annually. Underwater sensors provided temperature readings every 15 minutes at locations across the reef. Air temperature was also obtained during 2006 and 2007. Local tides, wind, weather, dolphin sightings, ship traffic, and oil offloading were regularly logged during fieldwork. Water samples were collected from across the reef and were shipped to Florida International University for water-quality analysis.

Hand grabs of surface-sediment samples were collected from the beach to just offshore of the reef to determine grain-size distributions. Sediment grain-size analysis was performed by manually sieving dried sediment hand grabs into size classes and calculating class weight percentages. The biogenic composition of approximately 100 sand-size grains from each sample was identified under a microscope. The number of coral grains, foraminiferal shells, coralline red algal fragments, gastropods, calcareous algae, echinoid spines, worm tubes, and other skeletal fragments was tabulated and SEDCON Index (SI) was calculated as a measure of reef health (Daniels 2005) where: P_c = number of coral grains, P_f = larger, symbiont-bearing foraminifera, P_{ah} = coralline red algae, gastropods, calcareous algae, echinoid spines, worm tubes, and other skeletal fragments and P_u - unidentifiable grains.

$$SI = (10*P_c) + (8*P_f) + (2*P_{ah}) + (0.1*P_u)$$

Data Analysis: Each year, the sharpest digital images were extracted from all transect and quadrat survey data to identify coral taxa and calculate percent benthic cover. We used a Red Sea comprehensive guidebook (Myers and Lieske 2004) to identify fish and coral taxa. Coral Point Count (CPCe) software (Kohler, 2006) was employed to evaluate coral coverage along each 10m transect by analyzing 25 consecutive frames representing an approximately 0.5 m x 2m wide area of coverage. Percent coral cover was calculated by identifying the bottom type at 60 randomly generated points projected onto each frame. Statistical analysis showed that data from 60 points was statistically identical to identifying the total coverage area from each photo. For each quadrat photo, the total surface area (cm^2) covered by each coral taxon was determined using CPCe software. Coverage analysis included identifying and counting fish, long- and short-spined sea urchins, and sessile organisms.

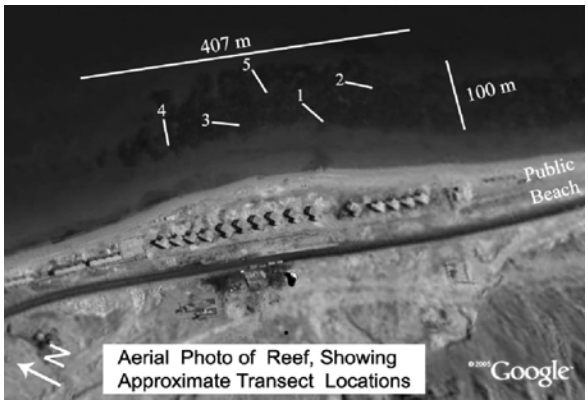

Figure 2: Satellite photo depicting Zaki's reef and the locations of five 10m transects monitored annually since 2004. Newly constructed villas can be seen on the reef's beach.

Results

Coral Taxa and Coverage: Each year percent bottom cover by coral and other parameters were compared to 2004 baseline survey data (Fig. 3). On average, of the 35 coral taxa identified in the Gulf of Suez, only six groups make up 94% of the coral cover on Zaki's Reef, with approximately 80% being hard and 20% soft corals. Dominant hard corals include *Acropora humilis, A. microclados,* and *A. hemprichii*

(staghorn), *Litophyton arboretum* (neptheids), *Stylophora pistillata* (finger), as well as *Porites columna* and P. *plantulata* (anemone) corals. On average, 25% of the coral (mainly *Acropora*) were dead in 2004. This value increased to 33% dead by 2007. Spatial patterns of coral cover and fish surveys indicate that offshore regions of the reef, where water is deepest, are typically healthiest and most diverse.

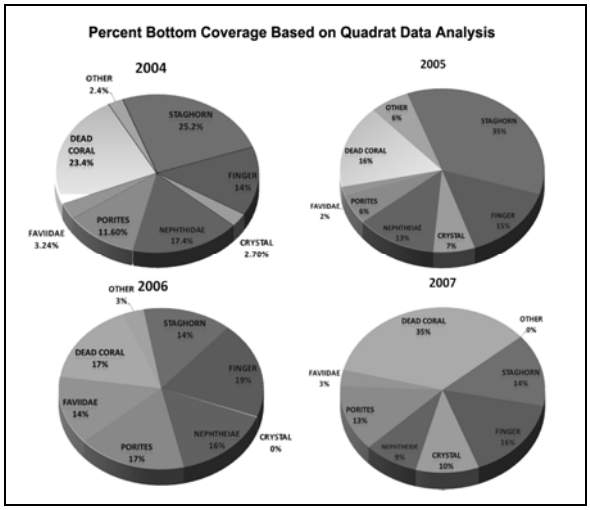

Figure 3: Percent change in coral cover over four years based on the random 0.5 m^2 quadrat survey 2004-2007. Note the increase in dead corals by 2007.

Averaged over four-years, random quadrat survey results indicate that *Acropora* spp. dominate (22.2% ±5.08 SE), followed by *Stylophora pistillata* (16.1% ±1.14 SE, *Litophyton arboreum* (13.5% ±1.92 SE), and *Porites* spp. (11.9% ±2.31SE). Mean dead coral coverage over the four-year study was 22.8% (±4.3SE). The remaining major groups, Faviidae (*Echinopora fructiculosa* and *Platygyra daedalea*, and *Galaxea fascicularis* (crystal coral), were roughly equally common, averaging 5.5% (±2.78SE) and 4.9% (±2.13SE), respectively.

Based on quadrat survey results, *Acropora* spp. were dominant (25%) in 2004 and increased to over 35% coverage in 2005. However, there was a huge decline for both *Acropora* (to 14%) and *Galaxea* (from 7% down to < 0.3%), between 2005 and 2006, yet there was no observed net change in percent dead coral that year (Fig. 3).

Video Transects. At all five transects, *Platygyra*, *Echinopora*, *Porites*, *Stylophora*, *Litophyton*, and *Acropora* corals were the dominant groups (Table 1). When 2007 coral coverage was compared to previous years, a net loss in coral was observed at Transects 2 and 4, while overall net increase was observed at the remaining three transects. The Kruskal-Wallis statistical test for population equality (Conover 1999) validated the null hypothesis that there was no significant difference in coral coverage at all transects over four years ($\alpha = 0.05$). Therefore, despite noted differences in species coverage between years (Table 1), differences remain statistically insignificant ($\alpha = 0.05$), indicating that as some species declined, others increased. Change in coral cover along Transect 3 over four years (Table 1) illustrate a shifting of corals as seen by a dramatic decline in *Platygyra* between 2004 and 2007. This was balanced by increase in *Porites*, *Stylophora*, and *Acropora*, while the overall amount of bottom substrate (dead coral and rubble) changed very little.

Table 1. Changes in percent coral cover at each 10 m- transect since 2004.

Percent (%) Change in Coral Coverage by Species at Each Transect					
	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5
Location on Reef	Nearshore S.	Mid-depth S.	Nearshore N.	Mid-reef N.	Offshore Mid-reef
	2005-07	2004-2007	2004-2007	2005-2007	2005-2007
Faviidae		3.87	-19.80	1.53	0.33
Porites	9.71	0.47	10.73	11.13	3.61
Finger Coral	1.98	-12.93	9.87	18.45	-1.19
Mushroom			0.27	0.80	
Crystal Coral				-32.34	5.27
Neptheidae	-0.08	0.13	-4.93	-15.30	-8.66
Staghorn	-1.64	-9.93	8.80	7.81	2.41
Leaf Coral		0.00			6.74
Diseased Coral		2.87	0.47	2.20	0.33
Total Change	9.96	-15.53	5.40	-5.73	8.83
Percent (%) Change in Bottom Substrate					
Rubble	-34.56	18.73	-11.73	-8.27	-9.10
Sand	21.33	-3.20	6.33	3.27	0.27
Ten meter transects surveys take across the reef each year indicate that Transects 1, 3, and 5 show an overall net gain in coral since the initial baseline survey in 2004 or -05, while Transects 2 and 4 reflect an overall decrease in coral and a greater increase in coral disease.					

Coral Diversity: The Shannon Weaver biodiversity index (Shannon, 1948) is an indicator of number of taxa and their evenness. Comparison over time from each transect and among transects indicated that biodiversity varied significantly between years in every instance ($\alpha = 0.05$) (Fig. 4).

Trends in this index varied depending on transect location and water depth, with the most marked increase in the index occurring at Transect 1 that lies furthest offshore. The greatest decreases in the biodiversity index occurred in 2006, one year after the oil-spill and the worldwide bleaching event.

Sediments: Sediment samples from across the reef indicate that grain-size distributions remained fairly consistent over time. In general, the finest sands are found in the reef trough and grade to coarsest offshore on the outer reef edge. Biogenic analysis of the sand fraction of each sample showed a change in the biogenic composition. Beginning with the 2005 data, the SI sediment index showed a statistically significant increase in coral fragments and fewer foraminiferal fragments.

Sea Urchins: Each year, long- and short-spine urchins were counted in quadrat photos with short-spine urchins being noticeably more abundant than long-spined taxa. While sea urchin population counts

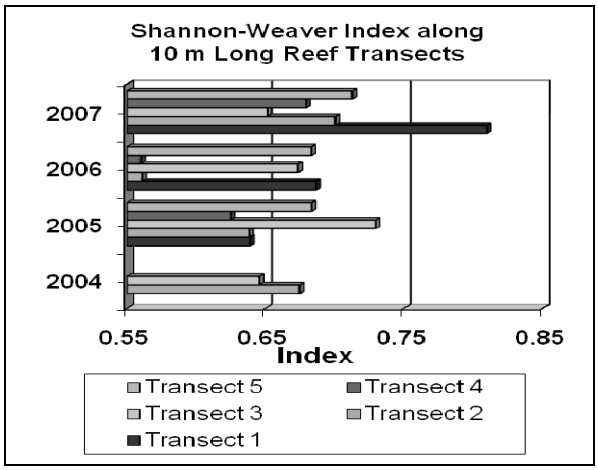

Figure 4: Calculated biodiversity index (Shannon Weaver) based on taxa present along five transects that range between 0.3 m and 3.0 m in depth.

were similar in 2004 and 2005, a notable, 42% increase in short-spine urchin was observed between 2005 and 2006, and a statistically significant increase of 58% between 2006 and 2007. No significant change was detected in the long-spined urchin population over the four years.

2005 Oil Spill: Immediate effects to the area in the days following the spill included increased turbidity, a clumping of oil in sediment and on rock faces, as well as a gummy residue on urchin spines. More than three years later, the effects of the 2005 oil spill were still widespread. Thick oil remained caked on rocks and the upper beach face, and was a few centimeters below the surface sediment on the reef and lower beach.

Temperature: Minimum and maximum daily air-temperature were 21ºC and 39ºC, respectively; an 18ºC difference. Daily underwater temperature range fluctuated between 0.3ºC and 4.5ºC. Seasonal water temperatures on the reef fluctuated widely between a low of 14ºC in February to a maximum of 34ºC in September. Shallower regions of the reef are markedly warmer and displayed greater daily variability than those in deeper water that experience stronger currents that allow for greater mixing.

Fish Bites, Coral Disease and Algae: Since 2004, annual digital photos documented a marked increase in fish bites, coral diseases, algae cover, and sedimentation on the reef. After 2006, an increase in parrotfish bites were observed on *Porites* corals, either territorial in nature, or possibly from remnant hydrocarbons in coral tissue attracting feeding (T. Goreau, pers. comm. 2008). Coral diseases were documented using photography beginning in 2006 and include *Porites* Ulcerative White Spot Disease and Pink and White Line Disease.

Water Quality: Average pH was 8.13 and average salinity was 44 ppt. Other analyses included total phosphorus (TP), soluble reactive phosphorus (SRP), total organic carbon (TOC), and dissolved inorganic nitrogen (DIN).

Discussion

At the outset of this research (2004), the reef was extraordinarily healthy and could be characterized as pristine. In 2005 a major oil spill occurred just three kilometers offshore of the reef during our field sampling. Although environmentally disastrous, it provided an opportunity to document reef conditions before and after an oil spill. This 2005 spill coincided with a widespread bleaching event (Lesser, 2007). Since 2005, new anthropogenic influences to this developing region of the Red Sea coastline included construction of a major shipping port, Port Soukna, less than 5 km north of the reef; a boom in seaside vacation homes within a few km of the reef; and the construction of villas on the reef's beach. In 2006, local fishermen staked claim on the beach at the southern portion of the reef for use as their port.

Table 2 summarizes known man-made and natural influences thought to be affecting this reef. The response of the reef community to recent stresses is mixed. In 2007, some positive signs of reef health were evident as three of the five monitored transects indicated an overall net increase in coral cover since 2004. On the other hand, since 2004, statistically significant differences were found in the amount of dead coral, urchin abundance, and biogenic components in the sediments. An increase in coral disease, fish bites, water turbidity, and sedimentation on corals, indicates a decrease in reef health.

Table 2. A subjective ranking of local influences affecting the reef.

Ranking of Current Stressors Affecting Zaki's Reef											
Stressor Scale		*Minor*			*Intermed.*			*Major*			
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Oil											■
Fishing/Fishermen				x							
Sedimentation								■			
Temperature				x							
Divers/Ecotourism		x									
Sewage/Pollution						x					
Swimmers		x									
Sea Level Rise	x										
Industrialization/Shipping									■		
Urbanization/Construction										■	
Low Tide Episodes		x									

Despite evidence that the reef continues to thrive, overall reef health deteriorated. Quadrat surveys showed a 50% increase in dead coral across the reef between 2006 and 2007. After the reef was stressed by the 2005 oil-spill and bleaching, corals appeared to be more susceptible to observed diseases and fish bites, similar to observations of Goreau and Wolf (2004). As petroleum hydrocarbons remain present in reef sediment, prolonged injury to this community

could continue for years and may explain much of the apparent decrease in overall reef health. The significant increase in short-spined urchins in 2007 is likely attributed to an increase in algae coverage.

We compared SRP and DIN in reef water samples to values reported in the literature (Lapointe et al. 1997). At nearly all locations, SRP levels were close to literature-reported values, while all DIN measurement, whose likely source is wastewater, exceeded literature's values by more than a factor of ten. The increased algal cover may be a response to these nutrients. Coral and mucus sampling for coliform bacteria were also positive.

Survival of this reef despite extreme environmental conditions clearly demonstrates its resilience over time. At the start of the study, results indicated that the reef was healthy and near pristine. Water visibility was high and sea urchin population counts were moderate. The sediment biogenic index initially suggested a healthy reef, and little coral disease was noted. Data from 2004/2005, prior to the oil spill, serves as a reference point, not only for this study, but also for any future coral reef studies in this region.

As man encroaches, human impact is no longer in question. Fishermen have relocated closer to the reef, beach houses that were under construction are being occupied, and with the new port, local shipping and oil offloading traffic has increased. Enormous tanker ships transiting less than 2 km away in very shallow water are displacing great amounts of sediment that appear to be contributing to reef sedimentation. The Gulf of Suez coastline to the north and south of Zaki's Reef is developing at an alarming rate without concern for the impact on the local environment.

Based on the existence of reefs in this region for the historic past, we can infer that select corals and their inhabitants can successfully thrive under extreme conditions. Corals and inhabitants here have adapted and appear more resilient to the large daily, seasonal, and interannual temperature variations and extreme salinities. Our results suggest that so far corals here are adapted to environmental variation and able to survive newly introduced stressors. Eventually, we anticipate that this reef will reach a point where it will no longer be able to recover from insults, and the community will succumb to the cumulative environmental impacts.

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Occupancy Models for Estimating the Size of Reef Fish Communities

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Abstract. Estimating reef fish species richness from underwater visual census (UVC) data has the potential to be negatively biased by non-detections of species present at a given site when the probability of detecting a species is less than one. To mitigate the effects of such sampling biases on subsequent ecological conclusions, we develop an occupancy model framework that estimates the probabilities of both detection and presence at locations in coastal Kenya using covariate logit-linear models. Based on a capture-mark-recapture (CMR) data structure for repeat observations over a closed sampling period, we find that reef-fish species characteristics are informative about differences in observed detection probabilities. Specifically, we show that schooling behaviour leads to higher probabilities of detection; that larger fishes are, on average, less detectable; and that detectability varies greatly by functional group. We also show how these characteristics of detection were poorly correlated with probabilities of occupancy and find that site-based covariates such as protection status may provide better models for presence. Finally we discuss the use of occupancy models in reef-fish surveys and suggest their use may be an important tool for reef ecologists in the future.

Key Words: mark-recapture; diversity; species richness; underwater visual census

Introduction

The diversity of coral reef fishes is unique in the marine environment and is among the characteristics that will most rapidly reflect fundamental changes in the ecological function of reef ecosystems. Although many alternate indices of diversity have been devised and used successfully in ecology (Buckland et al. 2005), species richness remains a prominent metric to describe the state of a given community (Kere and Schmid 2006). While species richness is frequently used to underpin the development of ecological theory, observing the true species richness of a given community is frequently difficult in practice (Dorazio et al. 2006) and, as a consequence of sampling error, the process signals contained in a given set of data may be weak or biased to an unknown degree (Allen and Starr 1982).

The potential for bias is particularly apparent in reef fish surveys where, for instance, small cryptic or large mobile species may not be readily observed due to low probabilities of detection in underwater visual census (UVC) data (Kulbicki 1998). Many reef ecologists recognize this issue and as a result the smallest and largest species on a given reef are frequently omitted from target species lists (McClanahan 1994). For the species that are present on target lists, detection probabilities are likely to be less than one (MacNeil et al. 2008b,a) and therefore fish species will have some probability of not being detected in a given survey, even over replicate transects at the same location. If detection probabilities are low, there may be many species present at a given location that remain undetected, negatively biasing the species richness observed to a substantial degree. Even though this issue is recognized conceptually, detectability is most often ignored during analysis and an implicit assumption is made that the probability of detection equals one.

Although most conventional sampling programmes do not address detectability directly in their design, model frameworks exist to address the issue of incomplete detectability during analysis. These models of species richness are based on capture-mark-recapture (CMR) models of tagged animals, whereby the probability of observing any given species on a particular transect is quantified relative to the detection histories of species observed during the survey, using a set of relevant covariates (Boulinier et al. 1998). Such an approach has been used successfully to estimate species richness in surveys of both birds (Kere and

Schmid 2006) and reef fish (MacNeil et al. 2008 a,b).

Although detection probabilities are important estimands in the quantification of species richness in UVC data, on their own they carry the implicit assumption that every species in the analysis was present at the sampling site during the census. For many species, this may not be the case, and the zeroes that arise in a set of UVC data can be due either to low probabilities of detection or because a given species was not present at a given sampling location. This second source of zeros has been called the occupancy state which, as it is either one (species present) or zero (species absent), can be quantified using presence-absence models combined with models for heterogeneous detection (MacKenzie et al. 2006). By combining models for detectability and occupancy, an analytical framework can be implemented to estimate species richness at a given survey location and, in the process, help to improve the ecological signal present in UVC data.

In this paper we use a well-known set of UVC data to demonstrate the conceptual utility of occupancy models to reef fish data. The statistical model presented describes separate components for handling the detectability and occupancy states of individual reef fish species, where covariate information from the species observed are used to estimate the proportion of a fixed species list that were not observed. The models are presented using a Bayesian inference framework.

Methods

To illustrate the applicability of an occupancy framework to reef fish UVC observations we selected data collected from coastal Kenya in the Western Indian Ocean (McClanahan et al. 2007). The collection of these data have been described elsewhere (McClanahan 1994) but briefly, the sampling scheme consisted of between 4 to 9 replicate UVC belt transects (5 m x100 m) conducted at four locations, two of which were protected from fishing (Mombasa; Malindi) and two of which were unprotected (Diani, Kanamai). Observations were made between 1992 and 2005, although sites were not surveyed in every year. The data were collected using discrete group sampling (DGS) where, depending on their physical and behavioural characteristics, fish from related groups or families were sampled on separate passes of a repeat transect in a given area. For these data, fish from eight families (Acanthuridae, Scaridae, Pomacentridae, Chaetodontidae, Pomacanthidae, Balistidae, Diodontidae, and Labridae) were counted to the species level using a fixed list of 161 potential species. Because the probability of losing or gaining new fish species among transects at a given site is near zero, the data permitted the mild assumption that the community was closed to species immigration and emigration over the sampling interval.

Occupancy models were built up from a conditional occupancy framework (MacKenzie et al. 2006) where, following conventional notation, y_i is the observed number of species detections accross k transects at a given site, ψ_i represents the probability of species i occupancy at a specific site, and θ_i represents the probability of detection for species j given that it is present at a particular site. The approach relates the occupancy state of species i to detectability using a two-part conditional model. First, if a species has not been observed ($y_i = 0$) then its occupancy state is unknown and can be described by

the sum of two conditional probabilities, the species being either not present ($\psi_i = 0$) or present ($\psi_i = 1$) but unobserved ($\theta_i < 1$):

$$p(y_i = 0) = \psi_i(1-\theta_i)^k + (1-\psi_i). \qquad (1)$$

Conversely, if the species is observed at least once over k transects ($y_i > 0$) then the record of events can be described as the bernoulli-distributed probability of occupancy times the binomial distribution of detections:

$$p(y_i > 0) = \psi_i \binom{k}{y_i} \theta_i^{y_i} (1-\theta_i)^{k-y_i}, \qquad (2)$$

for $y_i = 1, 2, ..., k$. Using these models as a basic structure for the detection of individual fish species, the framework can be extended to accommodate covariates of interest for ψ and θ using linear models and a logit link. For occupancy this is given by:

$$\frac{\psi_i}{(1-\psi_i)} = \beta_0 + \beta_1 x_1 + ... + \beta_j x_j, \qquad (3)$$

where the β's are covariate parameters for presence and similarly for detectabitliy:

$$\frac{\theta_i}{(1-\theta_i)} = \gamma_0 + \gamma_1 x_1 + ... + \gamma_j x_j, \qquad (4)$$

where the γ's are covariate parameters for detection. All of the γ's and β's are assumed to be $N(0,\sigma_j)$ distributed. Further details on this model and its implementation can be found in MacKenzie et al. (2006). Previous work on reef fish detectability has shown that schooling behaviour (schooling/non-schooling; SC); reef fish functional

group (FG; a grouping factor); and maximum total length (TL_{max}; a continuous variable) can affect probability of detection (MacNeil et al. 2008b,a). For simplicity, we used these three covariates in (4), the detection portion of our approach, to model the Kenyan reef-fish data and did not pursue covariate selection. In addition, we chose to include the same covariates in (3), as occupancy covariate selection has not been demonstrated previously and because our objective was simply to outline a form for the occupancy approach.

The resulting joint detection-occupancy model was implemented by Markov-chain Monte-Carlo (MCMC) simulation in the pymc (Fonnesbeck et al. 2008) toolkit for the Python programming language (Python Software Foundation 2006). Models were run for 15,000 iterations after a 15,000 iteration burn-in period and model convergence was assessed through visual inspection of parameter chains, where chains showing adequate mixing and stability were considered to have reached convergence.

Results

A range of between 34 and 84 species were observed across all transects, with consistently higher richness in the closed areas of Mombasa and Malindi. Observed occupancy proportions (Ω) were 23% or less in the open areas and were 34% or greater between the closed areas (Table 1). There was considerable year-to-year variation in richness values among sites, particularly for Mombasa which went from a high of 84 observed species in 1991 to a low of 55 observed species the following year.

Occupancy model convergence and mixing were good for all estimated parameters and all chains appeared to have reached convergence after the burn-in period. Posterior richness estimates ranged from 41% (66 spp.) to 74% (118 spp.) of the total species list, with estimates from 19% (31 spp.) to 41% higher than the observed proportions. There was slightly larger average estimated increases in richness in the closed (24%) versus the open (22%) areas. No sites showed evidence for all species being present, and the highest 95% credible interval bound lying at 83%.

Detection parameter estimates were highly variable among sites, with marked differences both between and among closed and open areas. Scrapevator detectability for example was relatively higher than site-averaged detectability in the closed areas, while being in the open areas. For other groups however (e.g. detritivores) detectability estimates were not consistent between management areas. The most detectable fishes tended to be grazers in the closed areas, and detritivores and invertivores in the open areas. Despite such evidence for differences among many functional groups, in many instances estimates spanned zero, suggesting additional covariates may be required to provide a satisfactory model of detectability at these sites.

Overall, the distribution of median estimated detection probabilities were more variable within open, as opposed to closed, areas. Kanamai in particular had a wider range of median detection probabilities across years relative to the other sites, with a somewhat diffuse distribution between zero and one that was apparent also in the Diani estimates. Conversely, the closed areas had more normally-distributed detection estimates, with median detectability values near 0.6 across years.

Unlike many of the detectability parameter estimates, fish characteristic parameter estimates were all poorly correlated with occupancy status, as almost all parameter estimates spanned zero. Exceptions to this included consistently lower planktivore occupancy in the open sites, and lower invertivore occupancy probabilities in Diani across all years. Although the covariate models were generally uninformative, the distribution of median estimated occupancy and detectability were consistently lower in the open versus closed areas. With the exception of Diani in 2006, the distribution of median estimated occupancy within sites was relatively consistent among years.

Median posterior estimates of detection and occupancy revealed distinct patterns in relation to median fish abundance per transect (Fig. 1). While sites with higher median abundance had clearly higher median probabilities of detection (Fig. 1a), median occupancy appeared (again with the exception of Diani in 2006) to be correlated with protection status rather than median transect abundance (Fig. 1b). To our knowledge, this has not been demonstrated previously.

Although detectability differences observed between open and closed areas are known to be highly correlated with abundance at a given sampling site (MacKenzie et al. 2006), probability of occupancy should be less so as a portion of this bias has already been expressed through the detection portion of the model. This appeared to be the case in our results, where only detection probabilities were shown to be highly correlated with median abundance at a given site.

Occupancy estimates on the other hand showed no such trend, instead reflecting unmodeled processes inherent in protection status that appear to have affected true species richness. Such processes may include effects of fishing on habitat quality that can reduce the occupancy probability of, for instance, coral-dependent species (McClanahan 1994). Indeed the lowest occupancy estimates consistently observed were for planktivorous species that, because they utilize the structural

complexity of the corals for shelter, are tightly linked to habitat conditions (MacNeil 2008) and likely to be directly affected by losses of reef habitat (Graham et al. 2008). The fine-scale resolution of species characteristics and their effects on detection and occupancy remain to be seen however, and we hope that the results presented here will generate new hypotheses in this area.

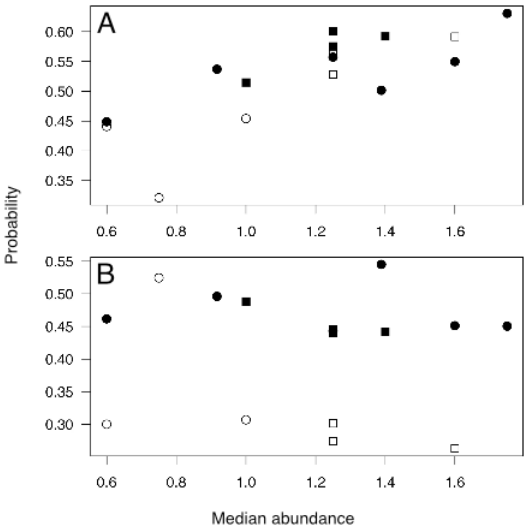

Figure 1: Median posterior detection (A) and occupancy (B) probabilities per median abundance of fishes observed per transect at Diani (open circle), Kanamai (open square), Malindi (filled square), and Mombasa (filled circle).

Given these results, we believe that occupancy estimates more directly reflect true trends in species richness through time than raw counts, as bias driven by the local abundance of fishes has, in part, been handled through modeling of detectability. Although these characteristics are evident in these data, it will require additional validation through both simulation and field studies to determine the efectiveness of occupancy models for informing management.

Discussion

We have outlined a general framework for estimating reef-fish species richness from conventional UVC data in which the issue of incomplete detection (a form of sampling error) is addressed directly using a conditional statistical model. The strength of the analysis is in the explicit parameterization of occupancy, where probabilities of presence can readily be linked to any potential covariates of interest on a given reef. While the occupancy models we developed from fish physical characteristics showed little correlation with occupancy probabilities, there is exceptional potential for occupancy models to link probabilities of presence with more likely covariates such as habitat characteristics (MacNeil 2008).

The covariates we chose, developed to deal with reef-fish detectability (MacNeil et al. 2008b,a) performed poorly in estimating occupancy, suggesting that fish physical characteristics are much more likely to affect detection probabilities than occupancy. If occupancy models are to be used routinely in reef-fish UVC surveys, covaraites must be identified that correlate appropriately with occupancy status. The presences or absences of particular reef fish species have been repeatedly linked to the site-scale habitats in which they are observed (Caley et al. 2001; Gratwicke and Speight 2005) and this suggests that reef-fish occupancy will most likely reflect trends in site-level characteristics. Although such an analysis has yet to be put into practice, our results show tentatively that protection status is a potentially informative covaraite, supporting the role of MPA's in promoting diversity.

That observed species richness is greatly affected by sampling bias due to the characteristics of the fishes observed has been widely recognized in reef fish surveys (Samoilys and Carlos 2000). Typically this is a downward bias (Ackerman and Bellwood 2000) that can, particularly where rare species are of interest, severely affect the conclusions drawn in a given analysis. In this example for instance, the highest and lowest observed species richness' in Mombasa were observed in subsequent years (1991-1992). Yet it is highly unlikely that there was a true loss of 29 species between those years followed by an increase of 9 species the year following. It is much more likely that low detectability of some of the rarer or more mobile species affected the observed counts across the transects within each year, leading to depressed counts in 1992. In contrast, the occupancy model estimates overlap substantially, indicating that there were likely between 113-121 species present at Mombasa in the early 1990's.

Given the consistent increases in observed species richness across all sites and years, using occupancy models to estimate true richness has substantial potential to reduce sampling error in a given set of data and, as a consequence, to increase the process signal strength that is inherent in the data itself (Allen and Starr 1982). Where, for example, the kinds of year to year variation discussed above are introduced to data through detection heterogeneity among species, the process signal of interest may become substantially weakened. Given that the most important questions for reef ecologists are ecological ones, these kinds of statistical estimators have the potential to structure some portion of sampling error in data,

while also providing ecological quantities of interest such as occupancy. These twin properties of reducing sampling variation and increasing signal strength suggest that occupancy models may see wide application in reef ecology in the near future.

Acknowledgements

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Table 1: Model estimates by site for observed (N) and estimated (N_{hat}) reef fish species richness from coastal Kenya; Ω and Ω_{hat} indicate observed and estimated proportion of total species list (161 spp.) at each location. Estimates are median values (± 95% credible intervals); status (open/closed) refers to fishing.

Site	Year	Status	N	Ω	N_{hat}	Ω_{hat}
Diani	1992	open	34	0.21	71(57, 87)	0.44(0.35, 0.54)
Diani	2003	open	34	0.21	72(55, 96)	0.45(0.34, 0.60)
Diani	2006	open	37	0.23	103(81, 120)	0.64(0.50, 0.75)
Kanamai	1992	open	35	0.22	66(53, 81)	0.41(0.33, 0.50)
Kanamai	2003	open	33	0.21	66(52, 90)	0.41(0.32, 0.56)
Kanamai	2006	open	37	0.23	73(58, 91)	0.46(0.36, 0.57)
Mombasa	1991	closed	84	0.53	123(113,133)	0.64(0.70, 0.83)
Mombasa	1992	closed	55	0.34	101(86, 121)	0.63(0.53, 0.75)
Mombasa	1993	closed	64	0.40	104(92, 117)	0.65(0.57, 0.73)
Mombasa	1998	closed	63	0.39	105(93, 117)	0.66(0.58, 0.73)
Mombasa	2003	closed	79	0.49	118(108, 129)	0.74(0.67, 0.80)
Mombasa	2006	closed	67	0.42	108(96, 119)	0.68(0.60, 0.74)
Malindi	1992	closed	67	0.42	108(96, 120)	0.68(0.60, 0.75)
Malindi	1996	closed	70	0.44	110(99, 122)	0.69(0.61, 0.76)
Malindi	2003	closed	70	0.44	113(102, 126)	0.71(0.63, 0.78)
Malindi	2006	closed	62	0.39	105(93, 119)	0.66(0.58, 0.74)

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Long-term changes in taxonomic distinctness and trophic structure of reef fishes at Cabo Pulmo reef, Gulf of California

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Abstract. Cabo Pulmo is the northernmost coral reef in the eastern Pacific, and has a remarkably high biodiversity. The Mexican government declared the area a National Park in 1995 and protection has been quite effective, resulting in increases in biomass and abundance of predator fish species. This study evaluates changes in average trophic level (indication of carnivory) and community structure of the 13 most important reef fish families, between 1987 to 2006. We performed censuses in observation cylinders (N=230), and calculated several ecological indices, as well as the taxonomic distinctness and average trophic level of the assemblages. The results show that fish community composition clearly differed in time as richness, diversity, evenness and taxonomic distinctness had higher values in the 1990s; at the same time the trophic level increased through the years, and its relation to most ecological indices was inverse and significant. The National Park had a positive influence on the fish community, at the same time carnivores seem to have depleted the abundance of many species and simplified the fish community. We conclude that conservation efforts have reached its goals but at the same time produced unexpected consequences in the composition and function of the area.

Key words: Community structure, Average trophic level, Marine protected area, México, Pacific Ocean

Introduction

The eastern Pacific region has many small and underdeveloped coral reefs, distributed from México to Colombia. The environmental conditions of this region are not adequate for zooxanthellate corals as the area presents relatively cold waters, large sandy tracts, a narrow continental shelf, and intense winter upwellings (Glynn and Ault 2000). However, the importance of those ecosystems in Latin America is high as they are characterized by an unusual mix of endemic taxa and immigrants from the central and western Pacific (Ketchum and Reyes-Bonilla 2001), and thus represent key areas for conservation (Aguilar et al. 2007; TNC 2008).

México is one of the countries that have paid substantial attention to research and protection of reefs, especially in the Gulf of California. In that inner sea there are only two areas where coral development is good enough that they can be considered as "functional reefs": San Gabriel Bay (24°N) and Cabo Pulmo (23°N; Reyes-Bonilla 2003). The latter has been recognized as a hotspot of marine species richness (Steinbeck and Ricketts 1941; Brusca and Thomson 1975; Robinson and Thomson 1992; Reyes-Bonilla 2003; Sagarin et al. 2008); the literature indicates more than 200 fish species and over 500 invertebrates reported for the area (Villarreal Cavazos et al. 2000; Hendrickx et al. 2005). The Mexican government recognized the relevance of the site and declared it a National Park in 1995, but the management plan was published until December 2006. According to the law of the country, all protected areas require at least some portion that should be open to extractive use (this means that the "no take zones" are not supported by the federal government, but nevertheless particulars or fishing cooperatives can exert its right to completely close any part of their own or concession areas; Lubchenco et al. 2007). However, at the same time the law establishes that no human use can be done in a reserve until the management plan is published (Aguilar et al. 2007). As a result of these regulations, we have witnessed the unusual case that *because of the lack* of a conservation program, the Cabo Pulmo residents, who were originally fishermen but changed their way of living to provide diving and sport fishing services, took the unilateral decision to avoid extractive uses in the park, and with help from NGOs and academic institutions, *de facto* managed efficiently the area for over a decade (Arizpe 2005).

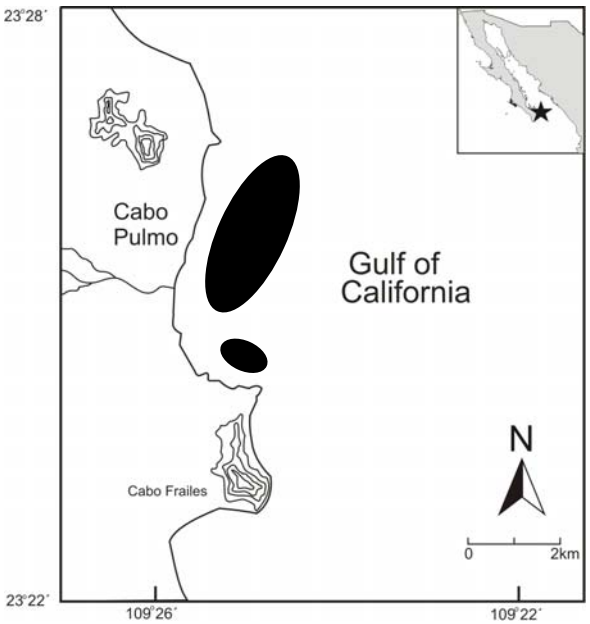

Figure 1: Study area, showing the approximate position of the coral reef.

There is a growing body of scientific evidence pointing out that no take areas bring noticeable advantages for conservation and fisheries, particularly higher biomass and larger fish sizes, an increase in abundance of carnivores (this is, a higher trophic level of the assemblages), and economic benefits for residents (Halpern 2003; Gell and Roberts 2003; Lester and Halpern 2008). Cabo Pulmo National Park is no exception and in the last decade there has been a noteworthy improvement in the general state of the reef and also in the living standard of the people (Angeles-Villa et al. 2008). Considering this background, the objective of this paper was to assess the effects of the conservation of the reef, taking as a measure the changes in trophic level and in several ecological indices of the fish assemblages at Cabo Pulmo. We noticed that as anticipated, a steady increase in abundance and size of carnivores has been occurring after the park was established, but interestingly, this also brought an unexpected reduction in the complexity of community structure. Our results call attention to responses at species and community level which were not anticipated when management efforts focused on specific targets (higher level predators), and resulted in an "ecological surprise" (Doak et al. 2008).

Material and Methods

Study area. Cabo Pulmo reef (Fig. 1) is located in the southeastern portion of the Baja California Peninsula, México. The area has a warm, tropical climate, and the sea temperature yearly fluctuates between 18° and 30° C (Arizpe-Covarrubias 2008). Photosynthetic pigment concentration is only 0.24 mg/m^3, with an annual range of 0.06 to 1.44 (NASA 2008), and correspondingly, nutrient concentrations are among the lowest in the Gulf of California, (WOA05 2008).

The coral community grows on basaltic outcrops and is composed of 12 species of five genera, with *Pocillopora* as the dominant one, followed by *Porites* and *Pavona*. (Alvarez-Filip et al. 2006). Coral cover was high in the 1980s and 1990s (over 30%; Reyes-Bonilla and Calderón-Aguilera 1999; Arizpe 2005) but decreased drastically after the 1997 ENSO event (Reyes-Bonilla 2001). To date recovery has been limited as the reef was impacted by several hurricanes and tropical storms, especially in 2003 (Alvarez-Filip et al. 2006). As a result, the coral cover in 2007 was 21%, a very small step up from the late 1990s.

Field Work. For this study we conducted censuses at Cabo Pulmo in nine different years during the interval 1987 to 2006. The counts were done at depths from 1 to 15 m (base of the reef), inside observation cylinders of 7.5 m radius in 1987 and 1994 (176 m^2 in area), and of 5 m radius in the remainder years (76 m^2). Because of the difference in sampling area and to make adequate comparisons, the number of individuals per census was scaled using density (ind/m^2) before any calculation.

For the analysis we included density data of the 13 "typical" reef fish families: Acanthuridae, Apogonidae, Blennidae, Chatodontidae, Haemulidae, Holocentridae, Labridae, Lutjanidae, Pomacanthidae, Pomacentridae, Scaridae, Scorpaenidae and Serranidae (Bellwood 1998; Robertson 1998), which are also the most abundant at Pulmo reef (Villarreal Cavazos et al. 2000). From that information we calculated the following community indices: species richness (S), Shannon-Wiener diversity (H´), Pielou´s evenness (J´), average taxonomic distinctness (Δ*) and taxonomic distinctness (Δ+). Finally, we estimated the average trophic level of the fish assemblages at each cylinder using the method of Pauly et al. (1998), and information of diet for each species from FishBase (2008).

Statistical analyses. We performed several statistical procedures. First, parametric analysis of variance and Tukey *a posteriori* tests were used to determine differences in each index among years in the sampling span. Then, the Pearson correlation coefficient was applied to evaluate the relation between trophic level and each ecological index, and simple linear regressions were conducted between trophic levels and those indices that were significantly related to the factor; in this case the idea was to depict the rate of change in the community structure using the slope of the regression (Zar 1999).

Results

The analysis of variance evidenced statistical differences in most indices (in all cases, degrees of freedom were 8,221). For richness, the year 1987 (19.8 ± 0.9 sp/census) differed from the rest (10.9 ± 0.8 sp/census general mean from 1994-2006; F=20.1, P< 0.01). In the case of density, again 1987 had the highest figure (2.7 ± 0.7 ind/m^2), but in addition, 2003, 2004, 2005 and 2006 had more individuals per census (in all cases over 1.0 ind/m^2) than years between 1994 and 2001 (less than 0.8 ind/m^2).

Diversity was highest in 1987 (1.77 ± 0.03 decits/ind), but the ANOVA and Tukey test determined that the difference among years was actually caused by the low values observed in 1994, 2002 and 2003, related to the rest (F= 4.1, P= 0.0001). Finally, evenness was significantly lower in 1994, very high in 1999, and remained statistically similar in the remainder years (F= 3.2, P= 0.001).

When the analysis covered taxonomic diversity (Δ+) there were no statistical difference (F= 1.7, P= 0.09) but in contrast, for Δ* (taxonomic distinctness) all years before 2000 had higher values than those of ensuing ones (F= 3.5, P= 0.001). The trophic level also differed among years (F= 3.1, P= 0.002), and 2002 and 2006 showed higher figures than all others (Fig. 2); furthermore, as a group the values of between 1994 and 1999 were significantly lower than those of the 2000s (t 228= 2.18, P= 0.03).

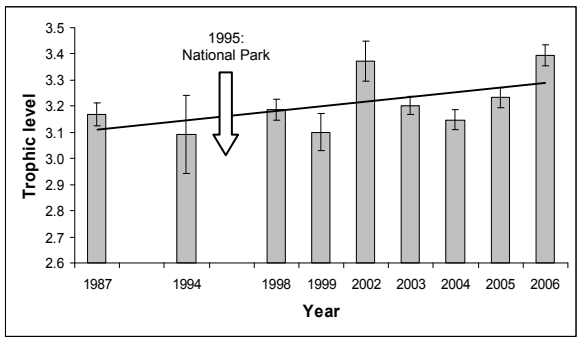

Figure 2. Trophic level of the icthyofauna at Cabo Pulmo reef, western México (average ± SE), and regression line of the values (slope: 0.008, ordinate: -14.65. r^2= 0.15, p= 0.16).

The data (Fig. 2) show that the trophic level has increased steadily from 1994 to 2006 (annual change of 0.008 units, according to the regression slope), and especially after the declaration of the National Park (annual change in 1999-2006 was 0.019); however, in both cases the relation was not significant, indicating that although the change seems to exist it is still not statistically relevant.

The correlation analysis between trophic level and the ecological indices (Table 1, Fig. 4) showed that the latter (including those with a taxonomic component), behaved similarly and were significantly linked. At the same time, it depicted that the trophic level was related negatively with all indicators, except fish density (positive relation) and Δ+ (not significant).

Table 1. Pearson correlation coefficients for ecological indices and trophic level of the reef fish community at Cabo Pulmo reef (1987-2006). All values are significant, except when noted (*). Key: S= species richness; N= density; H´= Shannon-Wiener diversity; J´= Pielou evenness; Δ*= Average taxonomic distinctness; Δ+= Taxonomic distinctness.

	S	N	H´	J´	Δ*	Δ+
N	0.33					
H´	0.63	- 0.21				
J´	0.06 *	- 0.49	0.79			
Δ*	0.18	- 0.05 *	0.29	0.22		
Δ+	0.30	- 0.01 *	0.28	0.13	0.53	
TL	- 0.15	0.19	- 0.35	- 0.35	- 0.14	- 0.05 *

The NMDS showed that community changed noticeably between 1987 and the 1990s, but afterwards (between 2003 and 2006), the composition of the assemblages became quite similar to what it was before artisanal fisheries were important.

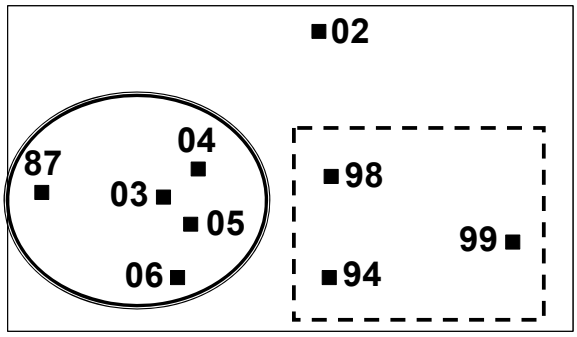

Figure 3. Nonmetric multidimensional scaling plot of the fish assemblages at Cabo Pulmo (1987-2006).

Discussion

This paper shows that as observed elsewhere, the establishment of Cabo Pulmo Marine Park led to fast changes (Polunin and Roberts 1993; Russ and Alcala 2004), and also modifications in the fish community. Practically all ecological indices showed a reduction in value with time (they were especially low in the early years of this decade), while at the same time the average trophic level of the community ascended gradually. These trends were clearly pointed out by the correlation analyses (Table 1) in which practically all coefficients had a negative sign, meaning an inverse relationship.

The effects of the increase in carnivore are better shown in Fig. 4, where simple linear regressions evidenced how a rise in trophic level is followed by a decrease in richness, diversity and taxonomic distinctness of the fish fauna, this is, by a simplification of the general community structure.

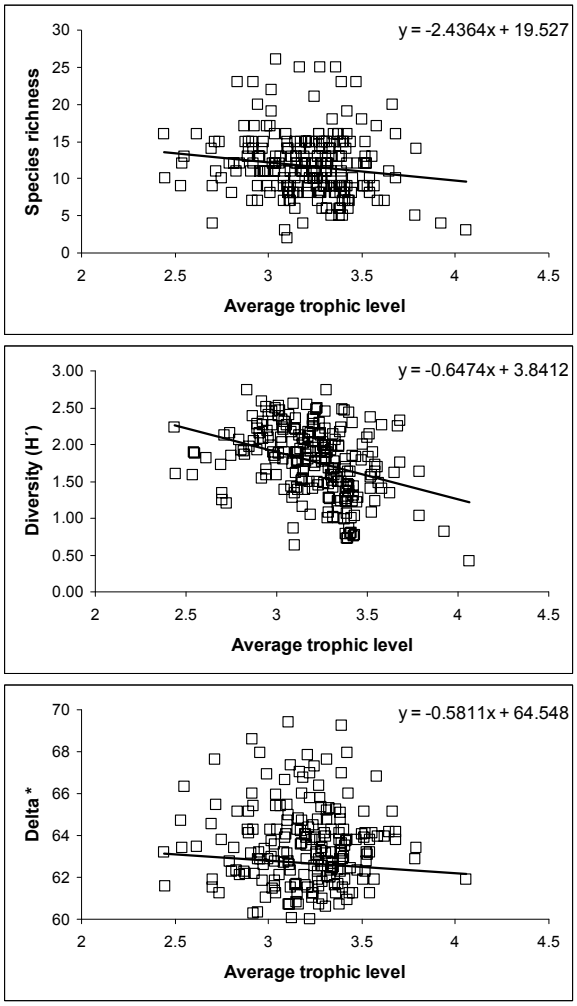

Figure 4. Regression analyses of trophic level and selected ecological indices of the icthyofauna at Cabo Pulmo reef.

Of those indices analyzed in Fig. 4, Shannon-Wiener diversity and species richness were most affected, as shown by the regressions slope. These measures depend on the number of fish taxa seen per transect and the relative abundance of the species (Magurran 2003), and consequently we suggest that the action of predators has been gradually diminishing the variety of fish types, and also the dominance of pomacentrids and labrids, the best-represented families in the reef (Alvarez-Filip and Reyes-Bonilla 2006).

In addition to the increase in trophic level, there are other indications of the good state of the assemblages. For example, the length of commercial species like the bumphead parrotfish *Scarus ghobban* Forskaal 1775, and the leopard groper *Mycteroperca rosacea* (Streets 1877), have augmented in the last years as a consequence of the lack of fishing (Fig. 5). Also, the larger size of fish aggregations is apparent, and finally the sightings of tiger and bull sharks in the reef have become common. All these observations point out the fact that large predators have returned to the reef.

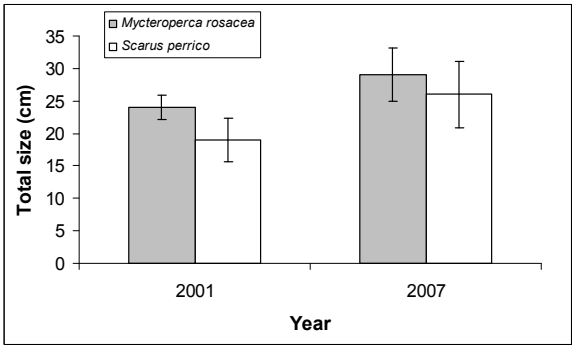

Figure 5. Differential in size (total length) of two key commercial species at Cabo Pulmo reef in the last decade.

The cited changes in fish assemblages are not a response to the local reef condition; to the contrary, coral cover has had small variations in the aftermath of the 1997 ENSO (average from 20% to 23%; Reyes-Bonilla 2001, 2003), especially because of repeated hurricane-caused damage (Alvarez-Filip et al. 2006). In addition, preliminary data shows that other faunas have been also influenced by the presence of higher level fish carnivores; for example, predatory damage is increasing on sea stars, and the abundance of certain species such as the urchin *Diadema mexicanum* Agassiz 1863, in 2006 was much reduced from the one in 1987. We suggest that the increase in fish predators is causing a substantial modification of the composition (and probably the function) of the entire reef ecosystem.

The response of the fish community of Cabo Pulmo to the increase in trophic level coincides with Barrett et al. (2007) remarks that predation is causing species specific and complex responses in Tasmanian reefs; however there is a key difference among regions because richness shifted in opposite directions as a consequence of the presence of large predators (down in México, up in Australia). In short, the divergent results advice of the need of more studies in different geographic realms before the trends of the ichtyofauna can be established with confidence.

We conclude that the establishment of Cabo Pulmo National Park has been a success from the perspective of the increase in abundance of carnivore species and sizes, and in general it can be said that the fish fauna shows an excellent conservation status. However,

there has been a simultaneous decrease in several ecological indices such as richness, diversity and taxonomic distinctness, an evidence of a simplification of the community, probably due to the depletion of specific kind of prey. Undoubtedly the conservation efforts have reached its goal, but nevertheless they brought upon unexpected changes in the ecological complexity and probably diminished the range of functions of reef fishes in this protected area.

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This paper represents the work of many students and colleagues who has visited Cabo Pulmo with us in almost 20 years of study. In particular we recived funding by SEP-PROMEP (Ref. UABCS-003, to HRB) and CONACYT-SEMARNAT (Ref.2002-C01-0189/A-1, to L Calderón-Aguilera, CICESE, Ensenada). LAF was supported by CONACYT (reg. 171864) and SEP Doctoral scholarships.

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Post-hurricane assessment (Hurricane Rita, September 2005) and recovery at the East Flower Garden Bank, Northwestern Gulf of Mexico

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Abstract. Hurricane Rita, a category 3 storm, passed within 83 km of the Flower Garden Banks (FGB) on September 23, 2005. Prior to that, in August 2005, Flower Garden Banks National Marine Sanctuary (FGBNMS) staff observed coral bleaching on the reef cap (18-23 m). In November 2005, we assessed hurricane damage and the effects of the bleaching event at the East Flower Garden Bank (EFGB) long-term monitoring site (Aronson et al. 2005). In June 2006, we conducted monitoring which included methods employed in November 2005. Hurricane Rita removed ~1.5% of coral colonies in repetitively photographed quadrats (mostly *Diploria strigosa*, *Porites astreoides*, and *Montastraea* spp.; sizes: 76 to 6,448 cm^2. Monospecific areas of *Madracis mirabilis* experienced catastrophic levels of breakage and toppling. Furthermore, ~10% of coral colonies (mostly *Montastraea annularis* species complex, *M. cavernosa*, and *Millepora alcicornis*) were bleached, an unprecedented bleaching level at the FGB since 1990 when ~5% of corals bleached (Hagman and Gittings 1992). Corals in deeper photo-quadrats (on the reef slope at 32-40 m) exhibited less bleaching than on the reef cap. The passage of Hurricane Rita caused water temperature to drop by 2-3°C, a hiatus in the prolonged thermal stress (> 30°C) which lasted 38 days. In June 2006, reef cap corals had largely recovered from the bleaching event of the previous summer, with bleaching closer to normal levels ~1%, while coral cover values and species relative abundance, remained stable.

Key words: Hurricane Rita, 2005, Gulf of Mexico, coral reef, damage, impact, bleaching, scouring, recovery.

Introduction

Hurricane-force tropical cyclones have frequently entered the Gulf of Mexico and traversed the East and West Flower Garden Banks (EFGB: 27°54'N; 93°35'W; WFGB: 27°52'N; 93°48'W) (Scholten and Deslarzes 1988). During the 20th century, severe hurricanes (Categories 4 and 5 on the Saffir-Simpson Index) may have moved through the FGB region (within ~250 km of the FGB) in 1900, 1909, 1915, 1957, 1961 (Category [Cat] 5), 1964, 1974, 1979, and 1980 (Cat 5). In August of 1980, Hurricane Allen, with a >5-m surge and wind speeds of 96 km/hr, caused physical damage to the coral reefs of the FGB long-term monitoring site (C. Combs, pers. comm., 1990).

The 2005 hurricane season in the Atlantic was the most active to date, fueled by record high sea-surface temperatures. Eleven tropical cyclones entered the Gulf of Mexico in 2005. On September 23 Hurricane Rita passed within 83 km of the FGB as a Cat 3 hurricane (Fig. 1); the other 10 storms passed more than 500 km away (The Weather Underground 2005).

Preliminary assessments of the FGB coral reefs following Hurricane Rita included substantial mechanical impacts, evidence of fractured and displaced corals (one dislodged colony was 4 m across and 2 m in height), sediment-scoured corals bordering large sand flats, and corals gouged and damaged from water-borne projectiles (Fig. 2, 3, 4). Impacts of Hurricane Rita at the FGB were preceded and followed by other natural disturbances: extreme seawater warming from August through November 2005 causing coral bleaching (48% of coral colonies were either partially or totally bleached) and the

offshore transport of river runoff from the Texas-Louisiana coast after the storm (NOAA CoastWatch 2005).

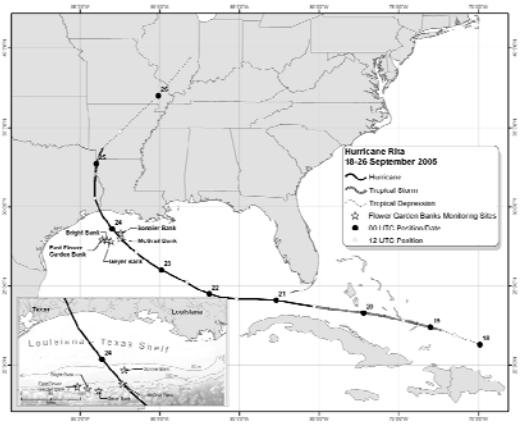

Figure 1: Track of Hurricane Rita, 18-26 September 2005 (National Hurricane Center 2006).

Figure 2: Sediment-scoured corals at the edge of a sand flat at the EFGB in October 2005. The scouring and sand removal were caused by the passage of Hurricane Rita on 23 September 2005. Photo by E. Hickerson, FGBNMS.

Figure 3: Coral colony gouged by waterborne projectiles during Hurricane Rita on 23 September 2005. Photo by E. Hickerson, FGBNMS.

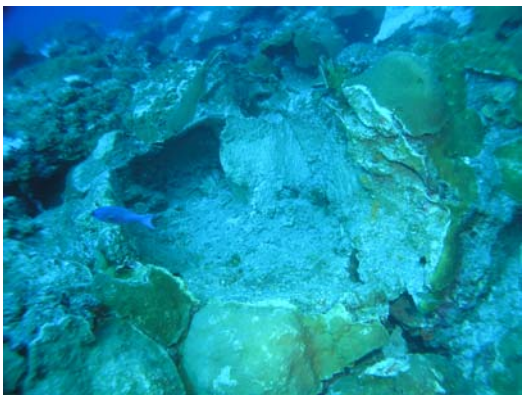

Figure 4: Footprint of a coral colony dislodged from the FGB reef cap by Hurricane Rita on 23 September 2005. Photo by E. Hickerson, FGBNMS.

In order to assess the impacts of both Hurricane Rita and the thermal stress on the benthic community at the EFGB, we made before-and-after comparisons of repetitive photographic quadrats, video footage of monitoring site perimeter lines, and of temperature and salinity data on the reef cap (turbidity, pH, DO, and Photosynthetically Available Radiation [PAR] data were lost to the storm).

Material and Methods

Repetitive Quadrats: To assess changes in the coral assemblage due to Hurricane Rita and bleaching, we photographed repetitive 8-m^2 quadrats within the 100 m x 100 m long-term monitoring site (18-23 m) and associated deep stations (32-40 m) in November 2005 (Gittings et al. 1992). The percent cover of benthic components was analyzed using CPCe® point-count software with Excel extensions (Kohler and Gill 2006) for repetitive photographs taken in June 2005, November 2005, and June 2006. Benthic components assessed included coral species cover, bleaching, paling, concentrated and isolated fish biting, and disease; as well as the cover of macroalgae, turf, and crustose, coralline and bare cover (CTB). We also used planimetry (Sigma Scan Pro 5®) to analyze selected corals within repetitive quadrats between June 2005 and November 2005. The goal of this analysis was to determine whether dominant framework-building corals of the FGB (*Montastraea annularis* species complex, *Diploria strigosa*, and *Colpophyllia natans*) grew or lost tissue laterally and to quantify the amount of coral cover lost due to the storm. Descriptive statistics were used to characterize the effects of Hurricane Rita within 8-m^2 repetitive quadrats.

Perimeter Lines: Perimeter lines around the 100 m x 100 m site were videotaped and a general sense of coral condition was obtained between June and November 2005. Divers videotaped two 100 m segments of the perimeter lines at the East Bank (north and east margins) in November 2005.

Perimeter lines were analyzed for hurricane effects such as toppling, removal, or breakage of coral heads, and thermal stress effects including coral disease, bleaching, paling, isolated and concentrated fish biting. These analyses were qualitative and therefore no statistical analyses were conducted.

Hydrological Data: We used YSI 6600 Series datasonde and a HoboTemp thermograph deployed on EFGB reef cap to examine temperature and salinity (specific conductance) data in June and November 2005. Storm damage done to the sensors prevented us from examining other data (turbidity, pH, DO, and PAR).

Results

Coral cover within repetitive quadrats on the EFGB remained high in November 2005 at approximately 61%, and species relative abundance showed continued stability with *Montastraea annularis* species complex, *Diploria strigosa*, *P. astreoides*, and *M. cavernosa* as the four dominant species (Fig. 5). Approximately 1.5% of coral colonies within repetitive quadrats were missing, most likely due to the effects of Hurricane Rita. This did not noticeably affect coral-cover estimates, which did not vary substantially from June 2005 (Fig. 5). *Diploria strigosa*, *Porites astreoides*, and *Montastraea* spp. comprised the majority of missing coral colonies with sizes ranging from 78 to 6,296 cm^2.

The most noticeable difference in the study site repetitive quadrats photographed at the East Bank in November 2005 compared to June 2005 was the level of bleaching: 9.74% (± 1.07 SE) in November and 0.57% (± 0.18 SE) in June. Most bleaching occurred on colonies of *Montastraea annularis* species complex, *M. cavernosa*, and *Millepora alcicornis*. Paling and fish biting measurements were low at 1.48% or less, and disease was not observed. In June 2006 bleached corals had recovered and bleaching levels were low (0.62% ± 0.24 SE), and little disease was documented consistent with November results.

From June 2005 to November 2005 algae group dominance changed. In June 2005 macroalgae was high at 24%, while crustose coralline and bare cover (CTB) was lower at 10%. After the hurricane, in November 2005 the inverse relationship between macroalgae and CTB was evident, with 24% CTB and 13% macroalgae (mostly turfs <3cm). Macroalgal cover is seasonally influenced and with the passage of Hurricane Rita it is likely that macroalgae were removed from the substratum (except short turfs). In June 2006 macroalgae and CTB were similar in cover (18 and 17% cover respectively), however, crustose coralline algae appeared to be higher in June 2006 (~11%) while the macroalgae group was dominated by *Lobophora lobata* instead of turfs.

Twenty-one coral heads from the 40 quadrats photographed within the 100 m x 100 m study site were removed between June 2005 and November 2005. Measurements of all missing corals were made from the June 2005 photographs to obtain a total area of coral cover loss. A total of ~3 m^2 of coral cover was removed from the 40 quadrats between June 2005 and November 2005; which amounted to a loss of 1.5% of the coral colonies in repetitive quadrats on the EFGB coral cap (18-23 m).

Among the nine reef slope (32-40 m) quadrats photographed in November 2005, 0.5% of coral colonies (2 colonies) were missing. There was also less bleaching (3.1%) compared to the reef cap quadrats. Coral cover remained high (74.5%) on the reef slope, and the dominant corals were *Montastraea annularis* complex, *M. cavernosa*, *Colpophyllia natans*, and *Diploria strigosa*.

Corals along the EFGB study site perimeter lines showed evidence of hurricane impacts, including the dislodgement, loss, or redeposition of entire coral heads as well as breaking of corals, and abrasion on the reef. There was evidence of bleaching and fish biting. Most distressed corals were affected by bleaching (6.4%), with slightly fewer incidences of fish biting (1.2%). As in June 2005, *Montastraea faveolata* and *M. franksi* were the most affected coral species. No evidence of disease was observed in November 2005. Physical damage was observed at several locations along the north perimeter line in November 2005. A partially bleached, dislodged colony of *Montastraea faveolata* was deposited along the perimeter line (Fig. 6). Several colonies of *Diploria strigosa* were either shattered or completely dislodged.

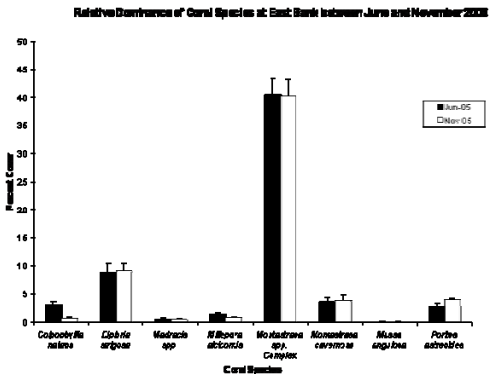

Figure 5: Relative dominance of coral species at EFGB between June and November 2005.

With the passage of Hurricane Rita, there was a sudden drop in temperature on the reef caps of the East and West Flower Garden Banks (Fig. 7). The temperature dropped from 29.6 °C at 0033 hours to 27.4 °C at 1933 hours on 23 September 2005. Temperature rose gradually after the passage of the

hurricane. By 0733 hours on 24 September, the temperature on the reef cap had reached 28.4 °C. During the passage of Hurricane Rita, mean daily salinity at the East Bank was 35.7 NTU.

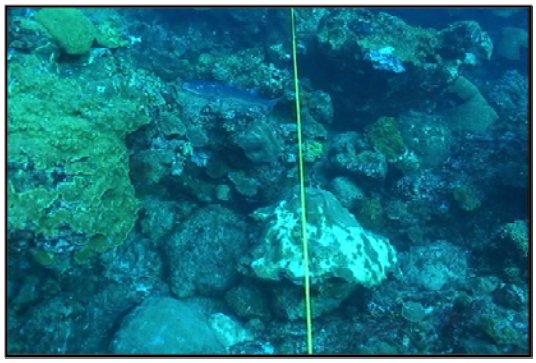

Figure 6: Photographs of a partially bleached *Montastraea faveolata* colony redeposited along the study site perimeter line.

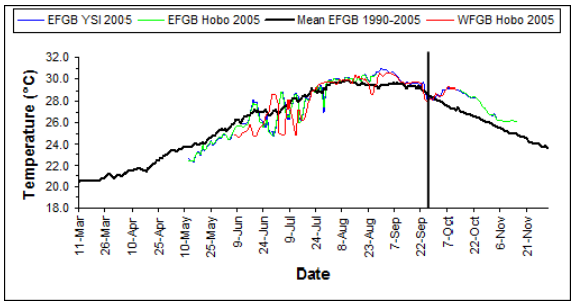

Figure 7: Water temperature at the Flower Garden Banks in 2005 and mean temperature at the EFGB from 1990 to 2005. Water temperature was measured using YSI 6600 Series datasondes (YSI) and HoboTemp recorders (Hobo). A vertical line drawn through the temperature profiles shows the timing of the passage of Hurricane Rita near the FGB (September 23, 2005). The 1990-2005 data are from Gittings et al. 1992, CSA 1996, and Dokken et al. 1999, Dokken et al. 2003, Precht et al. 2006, and Precht et al. 2008a.

Discussion

Mean daily significant wave height at NOAA data buoy 42019 was 4.5 m as Hurricane Rita passed by the EFGB. Depth, measured by the YSI instrument on the EFGB reef cap, rose from 23 to 25 m from 0300 hours on 23 September to 0400 hours on 24 September. The highest point of the storm took place at 2000 hours on September 23 when maximum wave height reached 5.9 m at buoy 42019. The intensity of Hurricane Rita at the FGB was evidenced by physical effects, including the large amounts of sediment removed from sand flats, the dislodgment of corals, and the gouging of corals. Current velocity and direction, measured by current meters placed near the sea surface on Texas Automated Buoy System (TABS) "Buoy V" located near the EFGB, and "Buoy N", located near the WFGB, provide another way to estimate the power of the storm. Buoy N recorded data up until 0830 hours on 23 September; current velocity at that time was 58.09 cm/sec. Current velocity was 57.6 cm/sec at 1100 hours at "Buoy V" (Bender et al., pers. comm., 2005). At the EFGB, water moved in a southeasterly and southerly direction at speeds ranging from 19.7 to 57.9 cm/sec from 23 to 24 September. The current direction recorded at the TABS buoys corroborates our observations at the EFGB of permanent stainless-steel station markers bent in a southerly direction.

In 2005, corals at the FGB were exposed to a prolonged period of thermal stress that was interrupted by the passage of Hurricane Rita. Thermal conditions on the reef cap were unquestionably more severe in 2005 than the average temperature at the FGB recorded to date. The temporary cooling of the reef cap as Hurricane Rita swept through the FGB region was likely the result of the mixing of cold deep water with the warm superficial water (the upper 50 m). At the foot of the FGB (~100 m), seawater temperatures average 19°C. Further down in the water column at 130 m, average water temperature is about 15°C (Nowlin et al. 1998).

Following Hurricane Rita 4 to 6 in of rainfall was recorded along the Mississippi and Atchafalaya rivers on 24 September 2005. Other areas along the coast of Louisiana experienced up to 12 in of rainfall on September 24. Satellite imagery of total suspended matter in the northwestern Gulf of Mexico showed that nearshore water associated with the high levels of precipitation was driven across the shelf and onto the shelf edge, including the area of the FGB. The discolored water seen reaching the FGB could have contained pollutants delivered directly into the northern Gulf of Mexico from industrial sites. Such pollutants include >0.14 km^3/yr of sewage effluent from municipalities, and routine discharges from petroleum refineries and petrochemical plants (Weber et al. 1992) and materials contained in 610 km^3/yr of discharge from the Mississippi and Atchafalaya Rivers (Dai and Trenberth 2002). Coastal and river-borne pollutants may include inorganic nutrients, organic carbon, insecticides and herbicides, and pathogens.

Hurricane Rita affected corals at the EFGB (18-23 m) in a number of ways. Approximately 1.5% of coral colonies photographed at the East Bank were missing from repetitive quadrat stations. *Diploria strigosa*, *Porites astreoides*, and *Montastraea* spp. comprised the majority of missing coral colonies, with sizes ranging from 0.95 to 80.61 cm^2. Approximately 0.5% of coral colonies at the deep stations (32-40 m) were missing. The field of *Madracis mirabilis*, which occurs at a similar depth to the deep stations, suffered severe breakage and damage during Hurricane Rita, but was recovering in June 2006 with new growth extending from the rubble. Corals within the deep

repetitive quadrats are mainly plating morphologies and, therefore, may not be as susceptible to breakage as more fragile, branching morphologies, such as *M. mirabilis* (but see Aronson et al. 1994).

Regional warming in 2005 produced coral bleaching at locations throughout the western Atlantic including Trinidad and Tobago, the BVI, Florida, and the FGB. Sea temperatures at the EFGB were elevated above 30°C, the HotSpot bleaching threshold for the FGB, for 38 days from 30 July to 8 September 2005. Although bleaching events are a natural occurrence, the increased frequency and severity of bleaching events is of concern because the likelihood of bleaching-associated mortality increases with exposure (Hoegh-Guldberg 1999). Additionally, higher temperatures have been linked to increased virulence of marine pathogens implicated in coral diseases (Harvell et al. 2002). We documented unprecedented coral bleaching at the EFGB in November 2005. Repetitive quadrats photographed at that time showed ~10% bleaching of the coral population, a marked increase from June 2005 (Hagman and Gittings 1992). Video perimeter results showed similar bleaching rates and similar differences in relation to video perimeter data taken in June 2005.

Despite the loss of corals, coral cover remained high on the reef cap (61%) and at the deep stations (74.5%). A comparison of shallow study site quadrats with deep station quadrats showed different bleaching patterns and hurricane impacts related to depth. Shallow quadrats had higher levels of bleaching (10%) and experienced a loss of 1.5% of coral colonies. The deep quadrats had lower levels of coral bleaching (~3%) and approximately 0.5% of coral colonies were missing.

The comparison of June 2005 with post-hurricane assessment data collected in November 2005 and June 2006 at the EFGB showed that coral cover within the established long-term monitoring study site remained relatively constant and species relative abundance showed stability with *Montastraea annularis* species complex, *Diploria strigosa*, and *Porites* spp. as the dominant species (Precht et al. 2008). In June 2005 macroalgae was high at 24%, while CTB was lower at 10%. After the hurricane, in November 2005, the inverse relationship between macroalgae and CTB was evident, with 24% CTB and 13% macroalgae (Precht et al. 2008a). By June 2006 macrolagal populations were reestablished while crustose coralline algae increased (Deslarzes et al. unpublished data).

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Stony coral species diversity and cover in the Florida Keys using design-based sampling

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Abstract. Large-scale sampling of stony coral species richness, species distribution, and cover was undertaken at 423 Florida Keys sites between Miami and SW of Key West during 2005 and 2007. A two-stage, stratified random sampling design employed belt transects to enumerate numbers of species and point-intercept surveys to quantify cover. The sampling design incorporated ten reef and hard-bottom habitats from < 1 m to 27 m depth, as well as oceanographic regions and areas inside and outside of protected management zones. These data provide insights into the spatial extent and factors influencing stony coral biodiversity. For stony corals, a pool of ~50 taxa encompassing the Orders Milleporina and Scleractinia, including species and morphotypes, was recorded. Significant differences were found in species richness and cover among cross-shelf habitats, with great values on inner shelf margin patch reefs, followed by deeper fore-reef slope habitats that extended to the 27 m depth limit sampled. In contrast, the shallow fore-reef, especially in areas historically dominated by the branching coral *Acropora palmata*, yielded relatively low numbers of species and cover that are presently dominated by smaller, brooding corals such as *Porites astreoides* and *Favia fragum*.

Key words: Benthic, cover, coral, habitat, species richness, stratified sampling.

Introduction

Coral reefs are in a state of decline worldwide from multiple stressors, including physical impacts, water quality changes, overfishing, disease outbreaks, and climate change (Pandolfi et al. 2003; Bellwood et al. 2004). Coral reefs in a degraded state are often characterized by one or more symptoms, including low abundances of top-level predators, herbivores, and reef-building corals with higher abundances of ahermatypic organisms such as seaweeds (Gardner et al. 2003). Like many coral reef ecosystems, the Florida Keys have experienced symptoms of "degradation" in recent decades, including declines in urchins and corals, particular *Acropora*, that have also occurred in the wider Caribbean (Dustan and Halas 1987; Chiappone et al. 2002). There are also a considerable array of natural phenomena affecting Florida Keys reefs such as atmospheric cold fronts, continental influence (Florida Bay-Atlantic Ocean exchange), and destructive tropical storms. This multitude of stressors makes it challenging to discern the degree to which human activities have comprised ecological integrity relative to natural variability (Somerfield et al. 2008).

Part of the uncertainty in understanding the factors driving decreases in populations of the coral reef ecosystem stems from the quality of the data used to document spatial patterns and temporal changes. Many historical studies lacked the statistical rigor necessary to adequately evaluate changes at the population-scale; in other words, the ecosystem area inhabited by a closed, interbreeding unit (Gardner et al. 2003). Generally, sampling has been at habitat-scales, that is, limited to a few reef sites within particular habitat types in restricted portions of the spatial domain (Dustan and Halas 1987; Porter and Meier 1992; Chiappone and Sullivan 1997). Frequently, selection of sampling sites within a given habitat did not follow standard randomization protocols, and consequently, the derived abundance metrics may not have been representative of the sampled habitats (Murdoch and Aronson 1999).

For the Florida Keys, we contend that the documented temporal changes and current views of spatial distribution and abundance patterns of coral reef benthos, particularly stony corals, are partly biased by the selection of particular reef habitats in particular locations that may not be representative of the larger ecosystem. For example, there is no doubt that areas historically dominated by *Acropora* corals, particularly the shallow (< 6 m) and deeper (8-15 m) fore-reef, have changed substantially, largely due to Caribbean-wide disease events (Dustan and Halas 1987; Chiappone and Sullivan 1997) and bleaching (Somerfield et al. 2008). However, debate has ensued for at least 25 years on the causes of coral reef decline (Porter and Meier 1992; Somerfield et al. 2008), thus making it tenuous for resource managers to determine

which courses of action to take to minimize localized threats in lieu of larger-scale factors such as climate change (Murdoch and Aronson 1999). In this short communication, we report on a large-scale sampling effort that encompassed hundreds of sites across the south Florida shelf to determine patterns of stony coral richness, species distribution, and cover. The 2005 and 2007 surveys were a continuation of previous efforts dating back to 1999 to quantify the abundance and condition of coral reef benthos throughout the FKNMS and built upon pre-existing data from hundreds of sites to guide the sampling design (Miller et al. 2002). Our purpose here is to illustrate the significant spatial variation in stony coral richness and cover, which has implications for reporting "average" reef status and underscores the significant inter-reef variability in this system (Murdoch and Aronson 1999; Somerfield et al. 2008).

Material and Methods

The Florida Keys are an archipelago of limestone islands stretching more than 360 km from near Miami to the Dry Tortugas, representing the only region of extensive reef development in the continental U.S. The islands are part of the south Florida shelf, a submerged Pleistocene platform 6-35 km wide and generally < 12 m deep (Lidz et al. 2003). The primary influences reef distribution and development are paleotopography and fluctuating sea level (Shinn et al. 1989; Lidz et al. 2003). Bedrock throughout the area is Pleistocene limestone, either exposed on the seafloor or lying underneath Holocene reefs and sands (Shinn et al. 1989). From inshore to offshore of the Pleistocene islands, a nearshore rock ledge extends ~2.5 km seaward and consists of hard-bottom, seagrass, and some inshore patch reefs (FDEP 1998). Further seaward is Hawk Channel, a broad trough-like depression dominated by non-coralline, non-oolitic grainstone, but also harboring several thousand patch reefs whose distribution is affected by the number and width of tidal passes (Marszalek et al. 1977). Bands of rock ridges exist further offshore along the outer shelf and on the upper slope from 30-40 m depth before tapering off into the Florida Straits. The semi-continuous reef tract is emergent in places, where Holocene reefs sit atop a Pleistocene coral ridge (~86-78 ka), forming a shelf-margin ledge (Lidz et al. 2003). Coral reef distribution reflects exchange processes between Florida Bay and the Atlantic Ocean (Marszalek et al. 1977; Shinn et al. 1989), which, is related to the size and orientation of the Pleistocene islands, the locations of major tidal passes, and the proximity of the Florida Current to the platform margin (Smith 1994).

At each site, four random sampling points per targeted site were generated in a GIS and located in the field using a differential GPS. Four 15-m transects were deployed per site. For stony coral species richness, an area 0.5-m out from each transect side was searched for the presence of any species present. Along the same transects, coral cover was estimated using the point-intercept method, in which the bottom type every 15 cm along the transect was recorded for a total of 400 points per site.

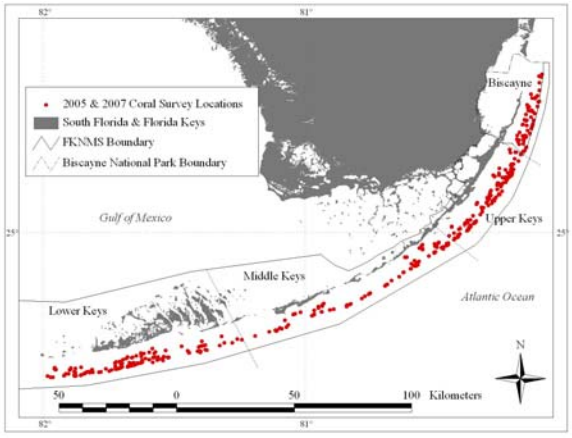

Figure 1: Survey locations sampled for stony coral species richness and cover during 2005 and 2007 in the Florida Keys National Marine Sanctuary and Biscayne National Park.

Table 1: Sampling effort for stony corals in the Florida Keys during 2005 and 2007. Available sites reflect the number of 200 m x 200 m cells with particular habitat based upon FDEP (1998) data

Habitat	Sites (% effort)	Sample area (m^2)	Sites available
Mid-channel patch reef (MPR)	87 (20.6)	5,220	3,532
Offshore patch reef (OPR)	69 (16.3)	4,140	1,243
Inner line spur and groove (IRT)	13 (3.1)	780	87
Shallow hard-bottom (LHBS)	39 (9.2)	2,340	972
High-relief spur and groove (HSG)	70 (16.6)	4,200	238
Patchy hard-bottom (PHBD)	32 (7.6)	1,920	1,247
Deeper hard-bottom (LHBD)	40 (9.5)	2,400	2,395
Low-relief spur and groove (LSG)	47 (11.1)	2,820	1,763
Fore reef 15-20 m (FRS20)	17 (4.0)	1,020	880
Fore reef 22-22 m (FRS27)	9 (2.1)	540	671
Sampling Design Total	423 (100)	25,380	13,028

Statistical estimation procedures for population abundance metrics (proportional transect frequency, cover) for a two-stage stratified random sampling design were adapted from Cochran (1977). Site species richness, species frequency of occurrence, and total coral cover were calculated for each site, and then pooled for sampling strata, consisting of

combinations of habitat, regional location, and management zone factors. Data reported herein emphasize habitat-related patterns. Statistical comparisons among habitats for mean site species richness, species frequency of occurrence, and cover were accomplished by computing confidence intervals (CI) based on the equation: CI = mean ± $t_{[\alpha, df]}$ *standard error. Standard errors were estimated by the two-stage, stratified random sampling design (Cochran 1977) and confidence intervals were adjusted for multiple comparisons using the Bonferroni procedure. The experiment-wise error rate was held at $\alpha = 0.05$ and the comparison-wise error rate was adjusted based on the number of multiple comparisons as follows: comparison-wise error rate = α/c, where $c = k(k-1)/2$ and k = number of categories (e.g. habitat classes).

Table 2: Physical characteristics of stony coral survey sites in the Florida Keys. Values are the ranges in transect depth (m), maximum vertical relief (cm), and site distance from shore (km). See Table 1 for habitat abbreviations

Habitat	Depth (m)	Max. vertical relief (cm)	Shore distance (km)
MPR	0.9-9.9	29-211	1.6-7.5
OPR	2.1-14.6	33-165	4.1-9.9
IRT	1.5-6.1	55-168	5.3-7.2
LHBS	2.7-7.0	20-92	5.5-9.6
HSG	0.6-9.4	32-253	5.9-10.2
PHBD	4.6-11.3	21-68	5.6-9.5
LHBD	5.7-13.7	10-80	4.6-10.6
LSG	7.6-16.2	14-98	5.5-10.7
FRS20	15-19.2	53-129	7.1-10.6
FRS27	21.6-27	84-144	6.4-10.3

Results

Surveys of the 423 Florida Keys sites yielded 49 stony coral taxa. Independent of region and management zone, mean (± 1 SE) stony coral site species richness (no. species/60 m^2) ranged from 12.1 ± 0.8 to 19.7 ± 0.4 among the 10 habitats (Table 3). Precision estimates (coefficient of variation) among habitats ranged from 2% to 7%. Stony coral species richness was positively correlated with mean percent coral cover (Pearson correlation coefficient r = 0.630), indicating that sites with greater numbers of coral species tended to yielded higher coral abundance. Among the 10 habitats sampled, maximum transect depth (r = 0.264) and distance from shore (r = 0.469) were only weakly correlated with site species richness, while maximum vertical relief was more highly correlated (r = 0.852). Species richness on mid-channel and offshore patch reefs was significantly greater ($P < 0.002$, Bonferroni-adjusted α) than on shallow (< 6 m), low-relief and high-relief fore-reef habitats. Particularly noteworthy was the low species richness of shallow fore-reef areas historically dominated by *Acropora palmata*. Although patch reefs yielded the highest mean site species richness, there was substantial variability from reef to reef, with species richness among mid-channel and offshore patch reefs ranging from 9-28 and 9-29 species per site, respectively. Deeper (6-15 m and 15-20 m) low-relief spur and groove habitat also yielded significantly greater numbers of species than shallow fore-reef habitats.

Mean stony coral cover ranged from 0.5% to 43.3% among the 423 sites surveyed (Table 3). There was substantial variability both among sites within habitats and among habitats. Mean coral cover was greatest on mid-channel (16.2%) and offshore patch reefs (8.5%), but was less than 8% for all other habitats. Relatively high coral cover on patch reefs was represented by massive framework species such as *Colpophyllia natans*, *Montastraea* spp., *Siderastrea siderea*, and *Stephanocoenia michelini*. Total coral cover was significantly greater on patch reefs ($P < 0.002$, Bonferroni-adjusted α) compared to low-relief low-relief and high-relief habitats on the shallow platform margin, and then increased again on the deeper fore-reef slope, especially below 15-m.

Table 3: Habitat summary of stony coral species richness (no. species per 60 m^2) and cover at 423 Florida Keys sites

Habitat (no. sites)	Richness	Cover
Mid-channel patch reef (87)		
Range	9-28	1.8-43.3
Mean ± 1 SE	19.7 ± 0.4	16.2 ± 1.3
Offshore patch reef (69)		
Range	9-29	0.5-22.5
Mean ± 1 SE	18.8 ± 0.6	8.5 ± 1.2
Inner line reef tract (13)		
Range	7-18	6.0-9.3
Mean ± 1 SE	12.1 ± 0.8	7.0 ± 0.6
Shallow hard-bottom (39)		
Range	8-18	0.8-3.0
Mean ± 1 SE	12.4 ± 0.4	1.6 ± 0.2
High-relief spur and groove (70)		
Range	7-21	0.5-13.8
Mean ± 1 SE	12.4 ± 0.4	5.1 ± 0.7
Patchy hard-bottom (32)		
Range	7-22	0.3-4.5
Mean ± 1 SE	15.2 ± 0.7	1.5 ± 0.3
Deeper hard-bottom (38)		
Range	7-25	0.5-13.0
Mean ± 1 SE	14.8 ± 0.6	2.8 ± 0.6
Low-relief spur and groove (49)		
Range	5-26	0.5-21.0
Mean ± 1 SE	16.1 ± 0.6	3.3 ± 1.2
Fore reef (15-20 m) (17)		
Range	14-24	0.5-21.0
Mean ± 1 SE	18.9 ± 0.7	7.0 ± 4.9
Fore reef (22-27 m) (9)		
Range	13-20	3.0-13.0
Mean ± 1 SE	16.3 ± 0.9	7.2 ± 1.1

Mean proportional transect frequency, or the percentage of transects where species were encountered, allowed for the partitioning of species into rare, common, and very common based upon habitat distribution and frequency of occurrence (Fig. 3). Relatively rare species (e.g. *Acropora palmata*,

Mycetophyllia aliciae, *Mussa angulosa*) were observed in few habitats, and when they did occur, were usually absent on 70+% of transects. Common species, which include many of the framework corals such as *Colpophyllia natans* and *Montastraea faveolata*, were present in most or all habitat types, but exhibited patterns in frequency of occurrence that were strongly habitat dependent (e.g. *C. natans* and *Solenastrea bournoni*). Very common species such as *Porites astreoides* and *Siderastrea siderea* were not only found in all habitat types, but frequency of occurrence values were also very high (> 75%).

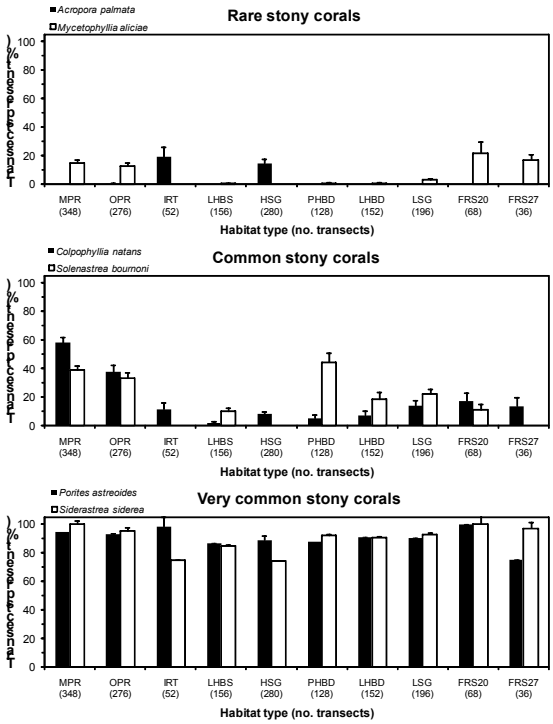

Figure 3: Mean proportional frequency (% of transects recorded) for selected coral species in the Florida Keys, ranging from very common to rare by habitat type. Error bars are +1 SE and numbers in parentheses on the x-axis are the number of 15-m x 4-m transects sampled in each habitat. See Table 1 for habitat abbreviations.

Mid-channel patch reefs were noted for their relatively high coral species richness and cover (Table 3). Figure 4 shows inter-reef variability in these two metrics for 51 mid-channel patch reef sites along ~200 km of the Florida Reef Tract, illustrating reef-to-reef variability, as well as regional variations. For both metrics, coral species richness and cover tended to be greater in the middle and lower Florida Keys, particularly in areas outside of FKNMS no-take zones, relative to the upper Keys and Biscayne National Park.

Discussion

Many biological phenomena are scale dependent, conclusions can be affected by the scale of observation, and caution needs to be exercised in scaling up results from small-scale studies to spatial and temporal patterns that were not sampled (Edmunds and Bruno 1996). Sampling at multiple spatial scales is usually necessary to determine whether patterns at one spatial scale are indicative of regional patterns (Murdoch and Aronson 1999). The interpretation of spatiotemporal changes in community structure is also made complex by biases introduced by site selection. For example, reefs with high coral cover, selected at the start of a monitoring program, can only remain unchanged or deteriorate once monitoring is initiated (Miller et al. 2002).

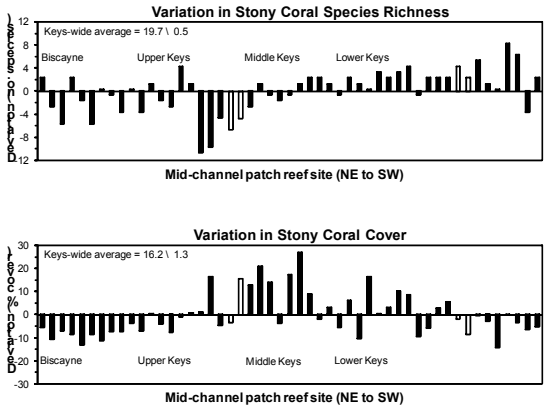

Figure 4: Deviations in stony coral species richness (no. species/60 m^2) (top) and percent coral cover (bottom) from domain-wide averages for Florida Keys mid-channel patch reefs. Open bars are sites within Sanctuary no-take zones.

The patterns and processes governing species richness and community structure are complex and scale-dependent (Murdoch and Aronson 1999. Species richness inventories can emphasize biodiversity hotspots, cold-spots, or the full gradient of species richness values. In addition, there have been attempts to identify indicator species whose occurrence patterns are correlated with the species richness of a larger group of organisms. For our Florida Keys study, taxon richness was measured in terms of the number of stony coral species identified in standardized search areas along belt transects sampled at each site. One of the most significant factors related to species distribution in the Florida Keys is habitat type, which reflects a combination of distance from shore, depth, and geomorphology (i.e. Pleistocene topography) (Shinn et al. 1989; Lidz et al. 2003). Greater numbers of coral species were recorded from inner shelf-margin patch reefs, followed by deeper fore-reef slope habitats that extended to the 27 m depth limit of this study. In other words, the greatest species richness of corals was recorded on either side of the main reef tract, including inner shelf margin patch reefs and offshore

of the main reef tract on the deeper fore-reef slope. In contrast, the shallow fore-reef, especially in areas historically dominated by the branching coral *Acropora palmata*, yielded relatively low numbers of species that are either widely distributed and frequently encountered (*Porites astreoides*) or species that are relatively rare in other habitats.

Relative to species richness, coral cover on Florida Keys reefs was more variable among sites within particular habitats, as well as among habitat types, but exhibited similar cross-shelf patterns to species richness. Coral cover was greater on patch reefs closer to shore and was significantly lower on the shallow platform margin, even on highly structured reefs where live *Acropora* cover was historically more abundant. Massive, mounding coral species dominated coverage inshore and some of these same species are prevalent on the deeper fore-reef, but not on the shallower platform margin. Previous large-scale surveys encompassing a large spatial area (Murdoch and Aronson 1999; Chiappone and Sullivan 1997; Miller et al. 2002) or timeframe (Somerfield et al. 2008) confirm the substantial inter-reef variability in the Florida Keys. Overlain on the geologic history of particular sites (Shinn et al. 1989; Lidz et al. 2003) and along-shelf position (Marszalek et al. 1977) are the responses of individual reefs to disease and bleaching episodes (Somerfield et al. 2008).

Cover and species richness are most frequently used with a focus on corals, because after all, corals are often the dominant organism or they are of high interest to managers. However, when coral cover is regionally low for most habitats as it currently is in the Florida Keys, a broader suite of metrics may be needed to evaluate ecosystem health and condition (Miller et al. 2002). In addition, there are so many potential indirect effects that might result from various management measures (e.g. no-take zones), in addition to larger-scale system variability, none of which can be predicted with any degree of certainty. Many previous and ongoing studies of coral reef community structure in the Florida Keys and elsewhere have focused on benthic cover as the abundance metric of choice for stony corals (e.g. Dustan and Halas 1987; Porter and Meier 1992; Somerfield et al. 2008). Benthic cover represents the net outcome of population dynamic rate processes such as colony recruitment, growth, and survivorship, whereas density and size structure, the two basic components of cover, provide information on the rate processes themselves, as well as on the net outcome. For example, a particular area with high densities of mostly small colonies versus another area with low densities of mostly large colonies may produce similar estimates of stony coral cover, but the two areas reflect very different demographic histories. Spatially explicit estimates of coral population density and size structure not only allow for tracking changes in abundance metrics over time, but can also serve as baseline data for subsequent studies of population and community dynamics (Smith et al. in press).

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Patterns in southeast Florida coral reef community composition

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Abstract. The Southeast (SE) Florida coral reef system is the northern extension of the Florida reef tract. This high latitude system lies offshore a heavily populated and urbanized coast and therefore is affected by numerous environmental and anthropogenic stressors. Using annual monitoring data collected in 2004, the southeast Florida reef community was analyzed to investigate patterns in community composition in various habitat types. Data was collected by SCUBA divers who conducted a $30m^2$ belt transect survey at 24 sample sites offshore Broward County (SE), Florida. Sites ranged in depth range from six to 18 meters. The 24 sites occurred on five different reef habitat categories: ridge-shallow, colonized pavement-shallow, linear inner reef, linear middle reef, and linear outer reef. These sites were established for the Broward County Board of County Commissioners and Environmental Protection and Growth Management Department, Biological Resources Division, in order to monitor Broward County coral communities and sedimentation rates in relation to possible effects from a beach renourishment project. The assessment took place prior to the renourishment project.

Key words: SE Florida, community composition, reef habitats

Introduction

Southeast Florida is comprised of Martin, Palm Beach, Broward, and Miami-Dade Counties. The reefs offshore southeast Florida are the northern portion of the Florida reef tract which ranges from the Dry Tortugas in the south to the St. Lucie Inlet in Martin County to the north. Early studies classified the system into three terraces running parallel to shore (first, second, and third reefs), each separated by sloping sand (Goldberg 1973). Recently, the SE Florida reef system habitats have been characterized in more detail (Moyer et al. 2003; Banks et al. 2007; Walker et al. 2008). From nearshore to offshore, reef habitats include: colonized pavement-shallow, ridge shallow, linear inner reef, linear middle reef, and linear outer reef. These habitats are generally deeper offshore with the colonized pavement-shallow and ridge shallow habitats ranging in depth from approximately 3m to 5m; linear inner reef ranging from approximately 6m to 8m; linear middle reef 12m to 15m; and the linear outer reef 15m to 20m.

Temporal and spatial variations exist in coral reef communities. In Florida Keys National Marine Sanctuary, the Coral Reef Monitoring Project (CREMP) documented stony coral cover declines between 1996-2000 (Callahan et al. 2007). Stony coral species richness was found to be greater on offshore patch reefs (Miller et al. 2002). For southeast Florida, offshore Broward County, species richness and evenness has been shown to increase on a north to south gradient, and *Montastraea cavernosa* was demonstrated to be the dominant scleractinian coral (Moyer et al. 2003). In 1973, Goldberg noted that the second reef (now classified as the linear middle reef) exhibited a greater abundance of gorgonians and stony corals such as *M. cavernosa* and *Dichocoenia stokesii* while the third reef (linear outer reef) had greater abundance and larger colonies of *Meandrina meandrites* and *Agaricia agaricites*. The processes and mechanisms that drive potential differences in community composition throughout the southeast Florida reef system remain unclear (Moyer et al. 2003).

The southeast Florida reef system exists within 3km of the coast offshore a highly urbanized area influenced by numerous impacts from commercial and recreational fishing and diving, major shipping ports, sewer outfalls, canal discharges, ship groundings, and marine construction activities. These reefs are important economic assets with an annual economic input for southeast Florida at over 5.7 billion dollars (Johns et al. 2003, 2004). The uniqueness, proximity, and value require characterization of the community, sustained monitoring, and increased investigations into limiting environmental/ecological processes.

Material and Methods

Data collected in 2004 from 24 sites was analyzed for this study. This year was selected for analysis because this was a period prior to planned beach renourishment dredging and construction activities. These sites were established for the Broward County Board of County Commissioners and the Environmental Protection and Growth Management Department, Biological Resources Division in order to monitor Broward County, Florida (southeast Florida) coral communities and sedimentation rates in relation to possible effects from the beach renourishment (restoration) project (Gilliam et al. 2005).

Each monitored site consisted of a permanent belt quadrat transect. Each transect was 20m long and 1.5m in width for a sample area of $30m^2$. The transects were marked with 21, 45.7cm long and 1.3 cm diameter stainless steel pins fixed in the bottom with marine, two part epoxy or Portland Cement, one meter apart (±1.0cm) in a straight line. The transects were placed in a generally north/south direction. The $30m^2$ transects were assessed by sequentially sampling 0.75 m^2 quadrats along both sides of the transect.

Each stony coral colony (scleractinian and hydrozoan, *Millepora alcicornis*) greater than 2cm diameter in the belt transect was identified (genus and species) and measured (±1.0cm). Two measurements were collected: live tissue length and width and whole colony length and width (which included dead portions and parts of the colony outside of the $30m^2$ belt transect). The number of branching octocorals (excluding *Briareum asbestinum* and *Erythropodium caribaeorum*) and fleshy sponges greater than approximately 2cm in width or height were counted. Community data calculations were: stony coral species density (colonies/m^2), stony coral percent live cover, stony coral whole colony size (cm^2), sponge density (colonies/m^2), and octocoral density (colonies/m^2).

Stony coral, octocoral, and sponge densities were determined by dividing the number of colonies in each transect by $30m^2$. Live tissue area of each stony coral species was determined by applying live tissue length and width measurements to the equation $A = L \times W$ (if $L \neq W$) or $A = \pi(L/2)^2$ (if $L = W$). The sum of all colony surface area values was divided by the entire transect surface area ($30m^2$) to calculate percent live stony coral cover.

Possible relationships among community data and habitat types (treatments) were investigated using multivariate (PrimerE, Clarke and Warwick 2001) and univariate (Statistica 6.0 (Statsoft)) statistical analyses. The sites occurred within five southeast Florida reef system habitat categories: colonized pavement – shallow (n=7 sites), ridge-shallow (n=4 sites), the linear inner reef (n=1 sites), the linear middle reef (n=6 sites) and the linear outer reef (n=6 sites) (Walker et al. 2008).

Community density parameters (three functional groups: stony coral, octocoral, and sponge), stony coral species density, stony coral percent live cover, and stony coral whole colony size (cm^2) data were analyzed. Multivariate analyses were completed on stony coral, octocoral and sponge density as a group along with stony coral species density and percent live cover data to examine differences across habitat categories. Site community data was square root transformed prior to the multivariate analysis and pooled into the five habitat categories (colonized pavement – shallow, ridge-shallow, linear inner reef, linear middle reef, and linear outer reef). Multidimensional scaling plots (MDS) (Clarke and Warwick 2001) were created using a Bray-Curtis Similarity matrix. MDS plots provide a visual representation (a "map") of the similarity (or dissimilarity) between sites such that the distance between sites in these plots is a measure of the relative dissimilarity in community composition. ANOSIM (analysis of similarities) (Clarke and Warwick 2001) tests were used to examine differences in stony coral species and community composition by habitat category. Sample site comparisons with R values of 1.00 indicated that the treatments were completely dissimilar while site comparisons with R values of 0.00 indicated that the treatments were completely similar. SIMPER (similarity percentage breakdown) (Clarke and Warwick 2001) analysis was used to determine which stony coral species or functional group (stony coral, sponge, or octocoral density) was responsible for driving the differences between treatments (habitat categories).

Univariate statistics were performed on the mean whole colony size (cm^2) of three important stony coral species in the system: *M. cavernosa*, *Stephanocoenia intersepta*, and *Siderastrea siderea*. Parametric analysis of variance techniques between habitat categories (ANOVA) and the Student-Newman-Keuls Post Hoc test between means (SNK) were completed after data was log transformed ($\log_{10}[x+1]$). Results were considered significantly different for values of $P < 0.05$.

Results

In 2004, within the 24 sample sites, 31 stony coral species were identified. Overall, the average site stony coral percent live cover was 2.1% ± 3.4% (mean ± SD) and average stony coral density was 2.6 ± 1.2 colonies/m^2. Stony coral cover was particularly high on two sample sites on the colonized pavement-

shallow habitat, FTL4 (16.9%) and FTL5 (14.2%). Octocoral density was 8.9 ± 13.9 colonies/m^2 and sponge density was 12.9 ± 9.4 colonies/m^2. Two of the most common stony coral species were *M. cavernosa* and *S. siderea*. *M. cavernosa* contributed most to overall stony coral cover (Table 1) while *S. siderea* had the greatest density. Table 1 shows *M. cavernosa*, *Porites astreoides*, *S. siderea*, and *S. intersepta* (common species in the area) mean site percent cover and percent of total stony coral cover.

	Mean Site % Cover	SD	% of Total Stony Coral Cover
M. cavernosa	1.7	4.2	53.3
P. astreoides	0.2	0.4	7.4
S. siderea	0.1	0.1	5.1
S. intersepta	0.1	0.1	2.8
Other	0.1	0.4	31.4

Table 1: Mean percent live cover, standard deviation (SD), and percent of total stony coral cover for *M. cavernosa*, *P. astreoides*, *S. siderea*, and *S. intersepta.* Other species include: *A. cervicornis, A. agaricities, Agaricia* spp., *C. natans, D. stokesii, D. clivosa, D. labyrinthiformis, D. strigosa, E. fastigiata, M. decactis, M. meandrites, M. alcicornis, M. faveolata, Mycetophyllia* spp., *O. diffusa, P. americana, P. porites, Scolymia* spp., *S. radians,* and *S. bournoni*.

Fig. 1 shows the MDS plot of community density (the three functional groups: octocoral, sponge, and stony coral) by habitat category. The ridge-shallow habitat was separated from all other habitat categories. This indicated that the ridge-shallow had a dissimilar community composition than the other habitat categories. Bray-Curtis similarity values showed that all sites were 60% similar and the ridge-shallow separated at the 80% level indicating that the ridge-shallow sample sites had more similarities with each other than the other habitat categories.

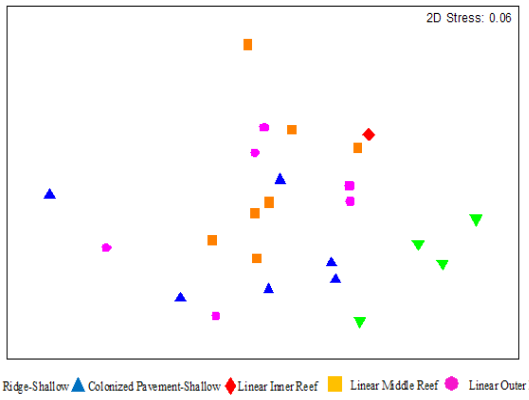

Figure 1: MDS plot of community density by habitat category. Each shape represents community density at one sample site.

ANOSIM results showed that the ridge-shallow community was clearly different but did overlap with the colonized pavement-shallow (R=0.405, p=0.029) and the linear outer reef (R=0.472, p=0.010) communities. The ridge-shallow reef community was well separated from the linear middle reef community (R=0.799, p=0.003). SIMPER results showed that sponge density was the functional group driving the differences (vs. octocoral and stony coral density) between the ridge-shallow and the other habitat categories with lower densities on the ridge-shallow sites (Fig. 2).

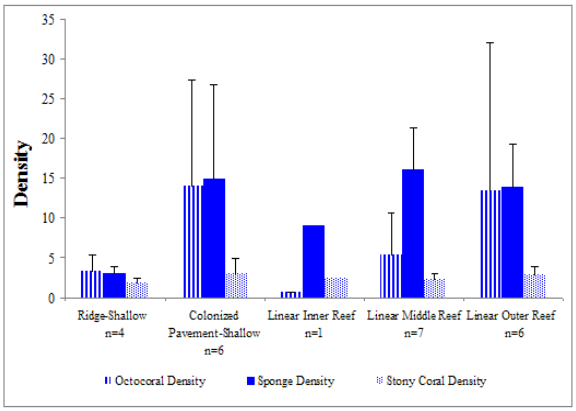

Figure 2: Octocoral, sponge and stony coral density by habitat category. Error bars represent +1 SD (n= the number of sites in each habitat category).

When analyzing each stony coral species percent cover and density in a multivariate manner, the ridge-shallow also had a dissimilar community composition compared to the linear middle and outer reefs. For stony coral percent cover, the ridge-shallow community was clearly different but did overlap with the linear middle reef (R=0.508, p=0.009) and the linear outer reef (R=0.528, p=0.005). SIMPER results showed that *M. cavernosa* was the discriminating species between the ridge-shallow and the linear middle and outer reefs. *M. cavernosa* had more cover on the linear middle and outers reefs compared to the ridge-shallow. *M. cavernosa* also had higher cover on the colonized pavement-shallow due to the two sample sites FTL4 and FTL5, but ANOSIM results did not indicate strong differences with the communities on the other habitat categories (Fig. 3).

For stony coral density, the ridge-shallow community was also clearly different but did overlap with the linear middle (R=0.664, p=0.003) and outer reef (R=0.687, p=0.005) communities. SIMPER analysis showed that the discriminating species in this case was *S. intersepta* because this species was absent on the ridge-shallow sites (Fig. 4). *M. cavernosa* had more colonies on the colonized pavement-shallow due to sample sites FTL4 and FTL5, but ANOSIM results did not indicate strong differences among the communities in the other habitat categories (Fig. 5).

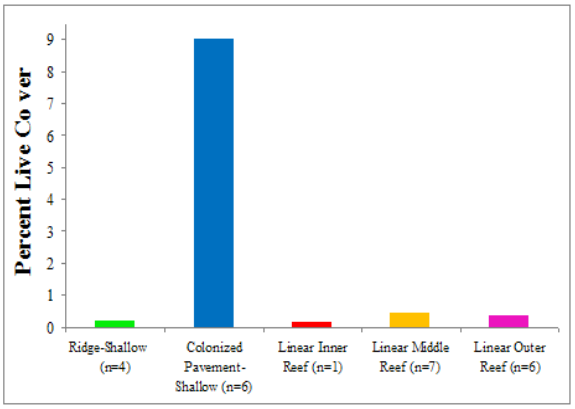

Figure 3: *M. cavernosa* mean percent live cover on all habitat categories. (SDs not shown are: Ridge-Shallow = 0.30, Colonized Pavement-Shallow = 8.02, Linear Middle Reef = 0.70, Linear Outer Reef = 0.23) (n= the number of sites in each habitat category).

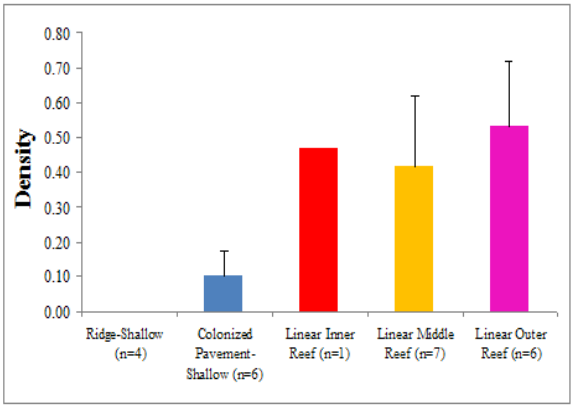

Figure 4: *S. intersepta* mean density for all habitat categories. Error bars represent + 1 SD (n= the number of sites in each habitat category).

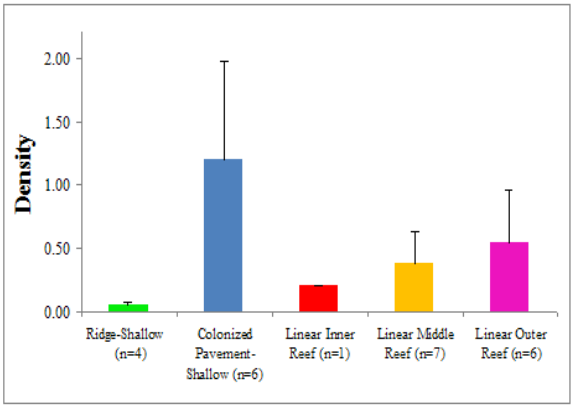

Figure 5: *M. cavernosa* mean density for all habitat categories. Error bars represent + 1 SD (n= the number of sites in each habitat category).

M. cavernosa had significantly larger mean whole colony sizes on the colonized-pavement shallow (Fig. 6). The largest colony in terms of diameter was a *M. cavernosa* colony (150cm) at sample site FTL4. *S. siderea* had significantly larger mean whole colony sizes on the linear inner, middle, and outer reefs and significantly smaller sizes on the ridge-shallow (Fig. 7). *S. intersepta* had significantly larger mean whole colony sizes on the colonized pavement-shallow and the linear outer reef compared to the linear inner and middle reefs (Fig. 8).

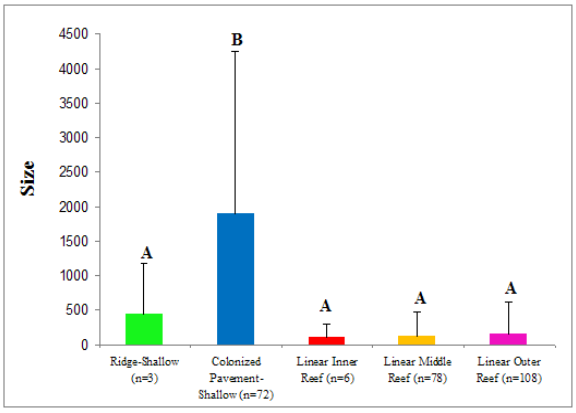

Figure 6: Mean *M. cavernosa* whole colony size (cm^2) for all habitat categories. Error bars represent + 1 SD (n= the number of colonies in each habitat category) (Differing letters indicate a significant difference between habitat categories).

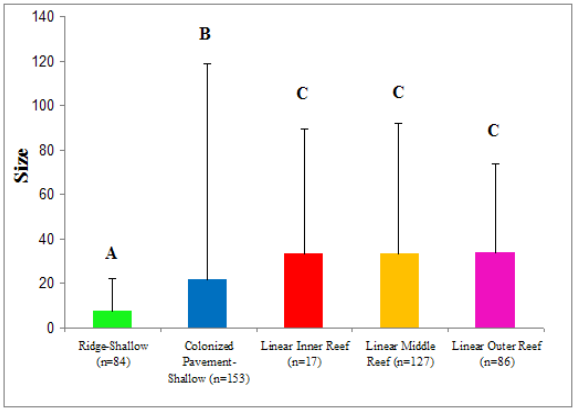

Figure 7: *S. siderea* mean whole colony size (cm^2) for all habitat categories. Error bars represent + 1 SD (n= the number of colonies in each habitat category) (Differing letters indicate a significant difference between habitat categories).

Discussion

The reef system offshore southeast Florida has greater octocoral and sponge densities than stony corals. Stony coral cover is generally less than 3%; however, there are exceptions with several colonized pavement-shallow sites (FTL4 and FTL5) having especially high cover (approximately 15%). The southeast Florida reef community varies by habitat category. With lower sponge densities and stony coral cover, the ridge-shallow has a dissimilar community compared to the linear middle and outer reefs. Three stony coral species (*M. cavernosa*, *S. siderea*, and *S.*

intersepta) are very common and important components of the system. *M. cavernosa* and *S. intersepta* have larger colonies on the colonized pavement-shallow. *S. siderea* have larger colonies on the linear inner, middle, and outer reefs.

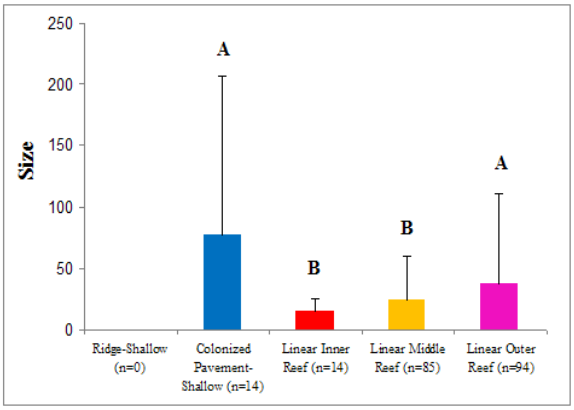

Figure 8: *S. intersepta* mean whole colony size (cm^2) for all habitat categories. Error bars represent + 1 SD (n= the number of colonies in each habitat category) (Differing letters indicate a significant difference between habitat categories).

The ridge-shallow and the colonized pavement-shallow habitats are part of a Ridge Complex that extends from Hillsboro Inlet (Broward County) south to Miami-Dade County (Banks et al. 2007). This complex is made up of sediments from cemented beaches and nearshore deposits (Banks et al. 2007). The linear reefs are made up of a Holocene *Acropora* framework (Lighty 1977; Lighty et al. 1978). According to this study, the inshore (ridge-shallow and colonized pavement-shallow) communities occurring on the cemented beach sand communities are dissimilar to those reef communities occurring on the Holocene *Acropora* framework (linear inner, middle, and outer reefs). The inshore communities are also more likely to be subjected to stress from re-suspension of beach sediments due to wave energy. It is unclear why the two sample sites on the colonized pavement-shallow, FTL4 and FTL5, have such high stony coral cover. Perhaps as Moyer et al. (2003) hypothesized, underlying substrate influences reef community composition. A complete understanding of the reef resources in SE Florida and what influences these vital communities is important for optimal management of a system that is subjected to so many stressors.

Acknowledgements

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Organisms associated with live scleractinian corals as indicators of coral reefs status in the Wakatobi Marine National Park (SE Sulawesi, Indonesia)

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Abstract. Organisms associated with live scleractinian corals were studied on four sites located around Hoga and Kaledupa islands in the Wakatobi Marine National Park. The number of coral colonies infested by coral associates was estimated along 20 m long line intercept transects and the number of coral associates found on each coral colony was recorded. A 0.5 m point intercept transect method was used to describe the benthic cover. A total of 2815 associates were recorded infesting 376 coral colonies. The most conspicuous coral associates were lithophagid bivalves making up for 73% of total coral infestations. The highest number of infested colonies was found for the genera *Montipora*, *Pavona* and *Porites*. They represented 33%, 23% and 18% of the total number of colonies infested respectively. The number of infested coral colonies and the density of *Lithophaga* spp. were high in the most impacted site (Sampela) and one of the intermediately impacted site (Pak Kasim's) whereas they were low in the most pristine site (Kaledupa). Despite the high biotic cover of Pak Kasim's, this site suffers from a similar level of infestation as Sampela suggesting process of reef degradation previously experienced by the most impacted site. Our results suggest that coral associates can be used as indicators of coral reef status.

Key words: Coral, Macrobioeroders, Coral predators, Reef health, Indonesia

Introduction

Coral reefs are critically important for the ecosystem goods and services they provide to maritime and subtropical nations (Moberg & Folke 1999). Reefs are currently in serious decline (Bellwood et al. 2004) due primarily to over-harvesting (Jackson et al. 2001), pollution (McCulloch et al. 2003), disease (Harvell et al. 2002), and climate change (Wilkinson 2004; Hughes et al. 2003). Already 20% of the coral reefs have been destroyed (Wilkinson 2004) and show no immediate prospects of recovery, 24% are under imminent risk of collapse through human pressures and a further 26% are under a long-term threat of collapse. The worst scenarios, prospected by Woolridge et al. (2005), suggest that reefs will become devoid of significant coral cover and associated biodiversity by 2050. The managing and a strong focus of key functional groups have become today a priority as part of insurance for sustainability (Hughes et al. 2003).

Coral associates (bioeroder and coral predator organisms) represent one of these key groups. Bioerosion and predation on scleractinian corals are indeed an important part of coral reefs dynamics. Scleractinian corals provide microhabitats and are used by a large number of parasites and other associated organisms, which use the tissue and skeleton of the coral colonies as food or substrata (Frank et al. 1995; Floros et al. 2005). Many taxa are involved and most of these coral associates stress the coral to some degree. Any natural or anthropogenic disturbances that lead to the loss of live coral tissue will ultimately increase the chances of bioeroder invasion. The bioerosion process can lead to important coral damage and even, depending on the intensity, can lead to mortality of coral colonies (Kleemann 2001). Bioerosion plays an important part in the degradation of the reefs and affects coral reef health**.** Nevertheless, although very important, the community structure of organisms involved in this process is relatively poorly documented. The aim of this study was therefore to link the different assemblages of coral associates on reefs around Hoga and Kaledupa islands in Indonesia with the health of these reefs.

Study sites and methods

This study was conducted on the reefs around the islands of Hoga and Kaledupa in the Tukang Besi Archipelago of the south-eastern coast of Sulawesi in the Banda Sea, in Indonesia and took place in July and August 2005. Four sites were studied (Fig. 1) and

were selected with a gradient of degradation (Table 1).

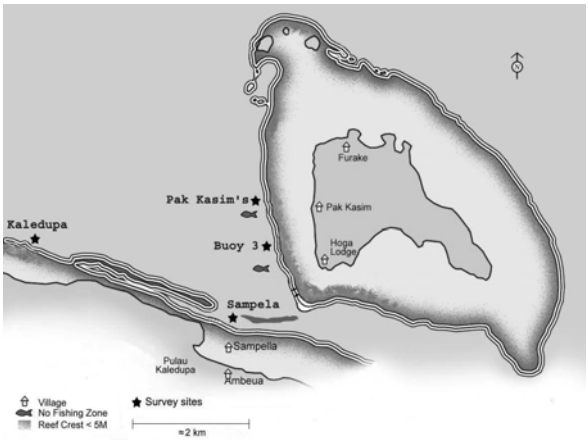


Figure 1: Location of the survey sites.

The number of coral colonies infested by coral associates was estimated along a 20 m long Line Intercept Transect (LIT) (English et al., 1997) at two different depths (6m and 12m). The number of macrobioeroders and coral predators found on each coral colony on the transect was recorded and corals were identified to the most precise level. To link the assemblages of coral associates observed with the characteristics of the benthic habitats, the cover of the major functional groups was estimated using a 0.5 m Point Intercept Transect (PIT) method (English et al. 1997) on the same 20 m-long transect as the macroinvertebrate survey.

	Kaledupa	Sampela	Pak Kasim's	Buoy 3
Latitude South	05°28'22''	05°29'01''	05°27'569''	05°28'40''
Longitude East	123°43'47''	123°45'08''	123°45'179''	123°45'45''
Rugosity	0.56 (0.08) N=25	0.73 (0.10)* N=15	0.58 (0.15) N=25	0.60 (0.19) N=23
Sedimentation rate (g d.wt.m^{-2}.d^{-1})	5.21 (1.01) N= 8	20.46 (2.12)* N= 9	7.25 (0.28) N= 6	
Light attenuation coefficient (K)	0.16 (0.01) N =5	0.24 (0.01) N = 4	0.12 (0.01) N = 5	0.13 (0.01) N = 5

Table 1: GPS position and characteristics of the different sites (* means that the difference with the other sites is signifiant, $p<0.01$).

Statistical analyses were performed with Minitab for parametrical and non-parametrical statistics. PRIMER v6 (Plymouth Marine Laboratory, Clarke & Warwick 2001) was used for analysis of community. Cochran tests were used to test for homogeneity of variances before ANOVA. Turkey's pairwise comparisons were used for post hoc comparisons. ANOSIM were performed to analyse similarities between sites after the ordinations (Multidimensional Scaling, MDS).

Results

A total of 831 scleractinian coral colonies belonging to 39 genera were recorded and analysed on 25 transects. Within live corals a total of 2815 associates were recorded infesting 376 coral colonies. The most conspicuous coral associates were lithophagid bivalves (2062 individuals infesting 242 coral colonies) making up for 73% of total coral infestations ; followed by dwelling hermit crabs of the genus *Paguritta* (306 individuals infesting 113 coral colonies) with 10.9% and the vermetid snail *Dendropoma maxima* (242 individuals infesting 116 coral colonies) with 8.6 % (Fig. 2 a and b). Other associates were less common and contributed little to total coral infestations (less than 3%).

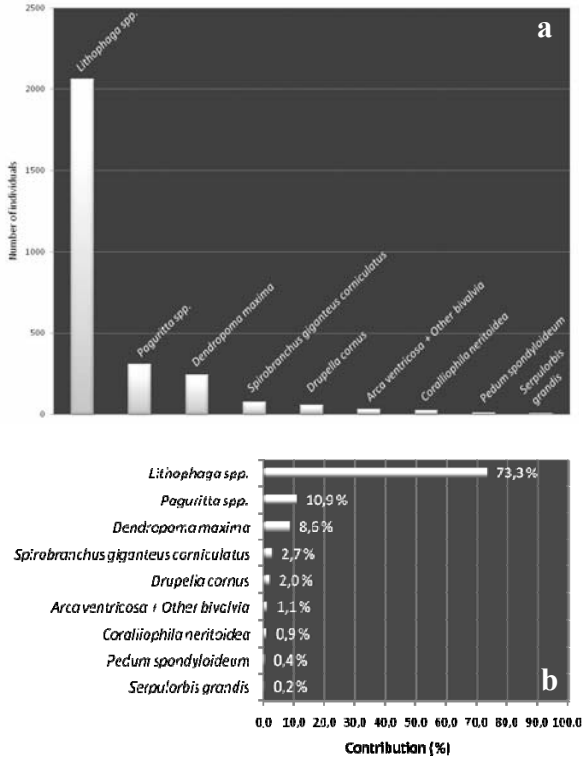


Figure 2: a. Number of bioeroder and coral predator organisms recorded during this study b. Repartition of the different bioeroder and coral predator species.

Infestation rate by scleractinian coral genus

The highest number of infested colonies on the transects was found for the genera *Montipora*, *Pavona* and *Porites* (Fig. 3a). They represented 33%, 23% and 18% of the total number of colonies infested respectively (Fig. 3b). These genera corresponded also with the most common taxonomic groups of scleractinian corals found on the transects (Fig. 3c).

The other genera were less common on the transects (Fig. 3c) and they represented less than 10% of the total number of infested colonies (Fig. 3b).

Infestation by site

At two sites, Pak Kasim's and Sampela, more than half of the coral colonies were infested (54% and 52% of the colonies for the two sites respectively). Only 32% of the coral colonies were infested in Kaledupa. Buoy 3 was in an intermediate state with 44% of the coral colonies being infested.

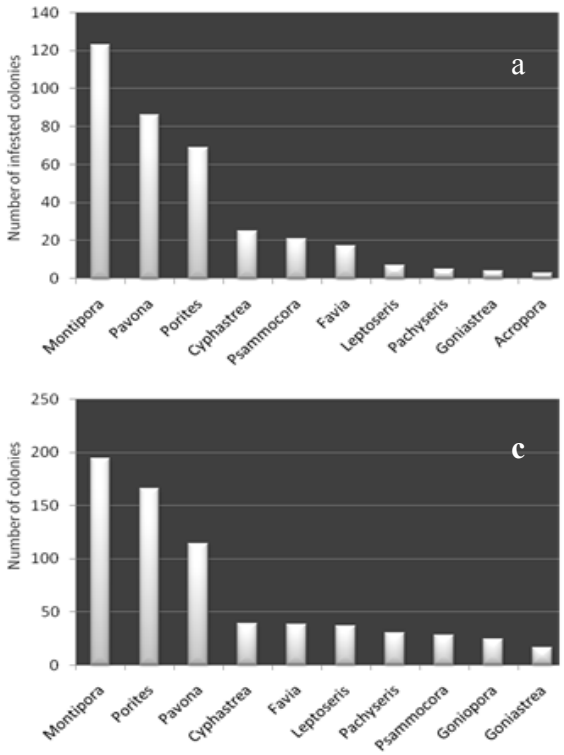
The majority of coral associates was found at all the sites excepted for *Drupella cornus* and *Serpulorbis grandis* which were absent at Buoy 3 and Sampela respectively. Within the same site, no significant difference was found between the two chosen depths (6 and 12 meters) in terms of the total number of infested colonies and the distribution of the studied organisms. A highly significant difference between locations (ANOVA One-Way, $F=18.42$, $p<0.01$) was noticed only for the lithophagid bivalves. The distributions of these organisms in Sampela and Pak Kasim's were significantly different from those in the other sites (Tukey's pairwise comparisons).

Community analysis.

Comparison of coral associates communities by Non-metric Multidimensional Scaling (MDS) and analysis of similarity (ANOSIM) indicated a significant difference among sites (ANOSIM One-way, Global R = 0.691, p=0.001)

Examination of the MDS plot (Fig. 4) showed a tendency of differentiation between the sites. Furthermore, pairwise comparisons of sites from ANOSIM resulted in R-values indicating important differences between Pak Kasim's / Kaledupa, Sampela / Kaledupa, Sampela / Buoy 3 (with R-values > 0.9) and between Pak Kasim's / Buoy 3 (R value > 0.8). No difference was found between the other paired sites. However, with regards to their positions on the MDS plot, the stations seem to be positioned along a gradient between two sites: Kaledupa and Sampela. Two groups were distinct on the MDS, the first one constituted by transects belonging to Sampela and Pak Kasim's, and the second one constituted by transects from Buoy 3 and Kaledupa.

Plotting the densities of the lithophagid bivalves on the MDS (Fig. 4), the global position of the replicates seems to be explained with regards to it. The site considered as the most "pristine", Kaledupa, has the lowest density of *Lithophaga* spp. (all densities < 1 ind.colony^{-1}). At the opposite, separated by the greatest distances on the MDS, are the replicates representing Sampela and Pak Kasim's. The Lithophagid'densities at these sites are the highest. The pattern observed, when the densities of this bivalve on the MDS are superposed, seems to be



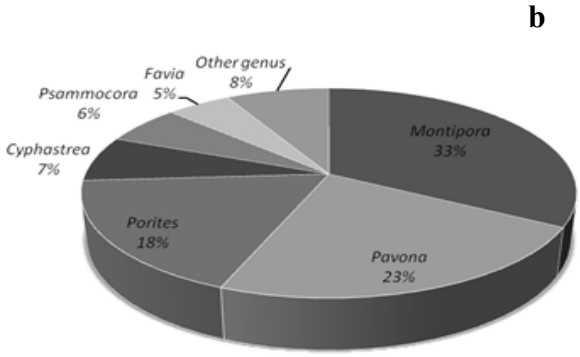


Figure 3: a. Number of scleractinian coral colonies infested for the ten most infected genera b. Ratio of the number of colonies infested corresponding to a given genus on the total number of infested colonies c. Number of colonies for the ten most common scleractinian coral genera.

coherent to the trend of the potential gradient between Sampela and Kaledupa.

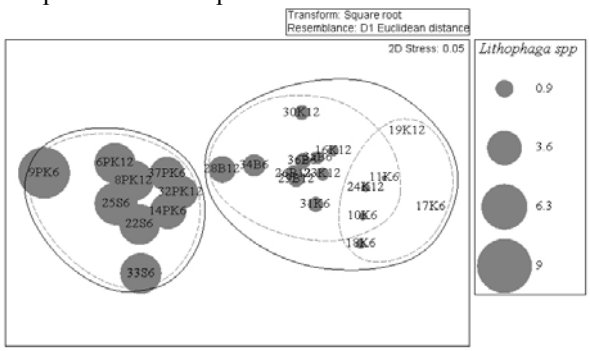


Figure 4: Bubble plot superposing the densities of *Lithophaga* spp. on the MDS (ind.coral colony^{-1}) (codes displayed on the MDS represent the name of transects and their characteristics: transect reference – localisation PK: Pak Kasim's, K: Kaledupa, S: Sampela, B: Buoy 3 – depth of the transect (6 or 12 meters)).

Link with coral reef status.

Significant differences between the studied sites were observed concerning the total benthic cover (multiple Kruskall Wallis tests on the different categories). Percent covers of abiotic and biotic categories in the four studied sites are illustrated in Fig. 5. For the same site, no significant difference concerning scleractinian coral cover was found between different depths. In contrary, for the same depth, significant differences were observed between the sites. The highest biotic (83%) and scleractinian coral cover (43%) was observed at Pak Kasim's. The same level of biotic cover was noticed at Kaledupa and Buoy 3 and no significant difference was observed between the three sites. However, the biotic cover was predominantly composed of soft corals at Kaledupa which explained a relatively low scleractinian coral cover in comparison with the other sites (20% and 26% at 6 and 12 meters respectively). Thus, the proportion of soft corals was significantly higher at this site. At 6 meter depth, the scleractinian coral cover at Kaledupa was significantly lower than that at Sampela (Kruskall Wallis test, $p<0.01$), despite the fact that Sampela was the site for which the biotic cover was the less dominant (only 39% of biotic cover). Abiotic cover at Sampela was thus significantly more important (Kruskall Wallis test, $p<0.01$). Moreover, concerning Sampela, the results of the LIT used for the infestation (126 colonies for three transects) put this site at the same level than Buoy 3 and Pak Kasim's (respectively 242 and 260 colonies for six transects) for the number of colonies recorded. The difference observed in the coral cover between Sampela and the other sites is attributable to the occurrence of a high numbers of small colonies at this place.

Regarding coral growth forms, multiple non parametric Kruskall Wallis tests showed that Kaledupa and Pak Kasim's had a higher proportion of branching corals. No significant difference was observed between the sites for the other categories of lifeforms taken into consideration (encrusting,

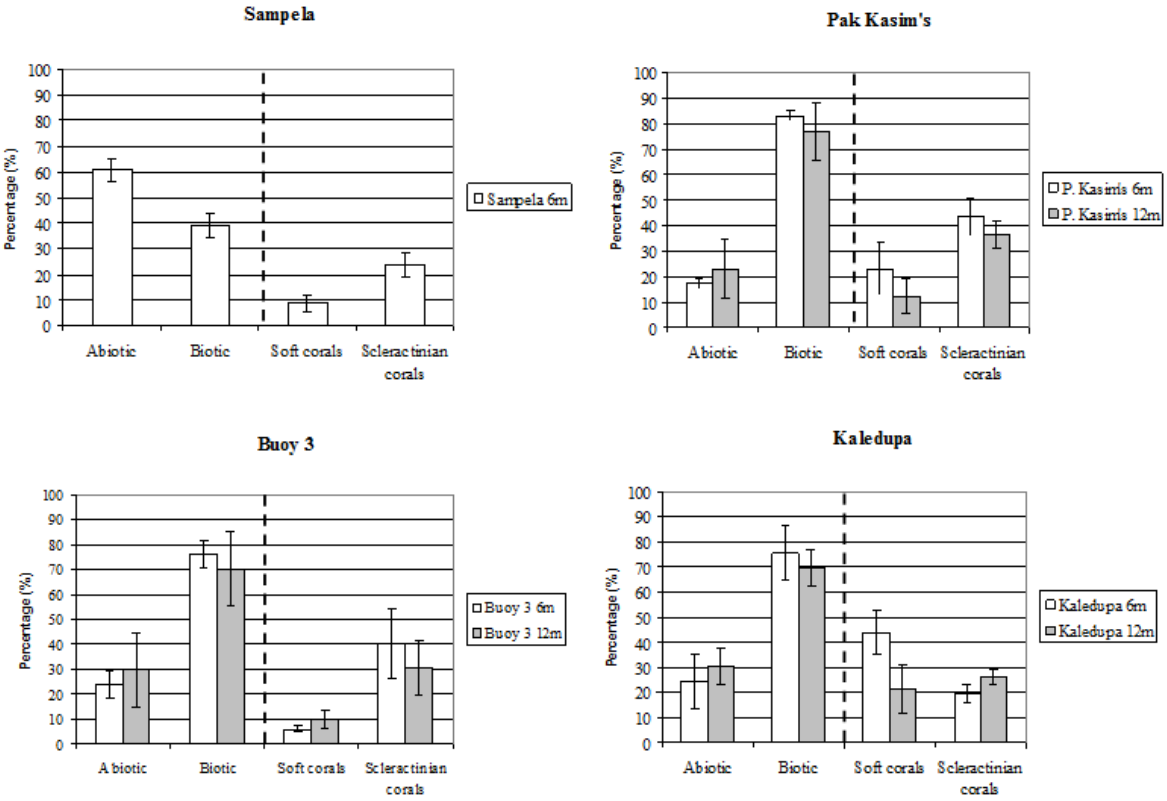


Figure 5: Summary of the benthic cover in the 4 studied sites (Sampela, Pak Kasim's, Buoy 3 and Kaledupa).

massive and foliose corals).

Discussion

When taking into account the environmental parameters measured such as sedimentation rate and rugosity, the obtained results suggest that Sampela can be considered as an impacted site, which agrees with the previous results of Crabbe and Smith (2002). In contrary, the sedimentation rate at Kaledupa was slighthy lower and reef complexity was higher than at the other sites. This site can be considered as a pristine one. The two other sites, Buoy 3 and Pak Kasim's, can be considered to be intermediately impacted.

Analysis of bioeroder and coral predator communities showed that despite the lack of any significant difference in biotic cover between the three sites Kaledupa, Buoy 3 and Pak Kasim's, a common gradient seems to emerge. The superposition of bioeroders' densities on the MDS plot showed the essential role played by the boring lithophagid bivalves, which probably contributes to the similarity between Sampela and Pak Kasim's. Although the biotic and coral cover of Pak Kasim's is high, this site also suffers from a similar level of infestation and perturbation as Sampela. However, unlike Sampela, visual observations and monitoring methods at Pak Kasim's did not reveal a substantial number of dead corals infested by bioeroders as in Sampela. It suggests that this site is going through the process of reef degradation previously experienced at Sampela.

The study of organisms associated with live scleractinian corals in the Wakatobi Marine National Park has shown that infestation rate of coral colonies by coral associates (essentially boring lithophagid bivalves) seems to be related to coral reef status (pristine, intermediate and impacted sites). The infestation rate by coral associates is more important in impacted site than in pristine site. Almost all coral associates are filter-feeding heterotrophs and hence, would be expected to increase in numbers in water with elevated nutrient concentrations (Risk et al. 2001; Floros et al. 2005). In consequence, as suggested by Risk et al. (2001) the health of a reef may be evaluated by scouring the density of coral associates on massive corals. This is base on the theory that coral associate numbers will increase with organic loading: stressed corals will be less able to protect themselves from settlement and overgrowth (Risk et al., 1993).

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Elkhorn Coral Distribution and Condition throughout the Puerto Rican Archipelago

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Abstract. We estimated 673 km^2 of potential habitat for *Acropora palmata* in Puerto Rico using GIS and measured the distribution, abundance and condition with a random sampling approach at six sites (3 east and 3 west coast MPAs). Average density ranged from 0.2 to 9.8 colonies/100m^2 and was highest on west coast sites (Rincón and Cabo Rojo) and these sites showed lower predatory snail (*Coralliophila abbreviata*) prevalence and density. Overall, the prevalence of white band disease was low (2.4% of colonies) yet variable and recent mortality due to diseases was highest in Culebra. Mortality caused by boring sponges (*Cliona tenuis*) was only observed at west coast sites. Nine plots were established in 2007 for long-term monitoring of demographic variables using individually tagged colonies. The results indicate that spatial variability must be considered when assessing trends at the regional level in order to clearly understand population status and responses to management actions.

Key words: Puerto Rico, *Acropora palmata*, spatial distribution

Introduction

In 2006, the corals *Acropora palmata* and *A. cervicornis* were listed as threatened species under the US Endangered Species Act (NOAA 2006), underscoring the need for assessment of status and recovery of their populations. An important realization during the assessment and listing process was that trends in the populations of Puerto Rico and demographic information necessary for modeling changes at the population level was unavailable.

In Puerto Rico the spatial extent of Elkhorn coral (*A. palmata*) and its condition throughout the archipelago was described by Almy and Carrión (1963) and Goenaga and Cintrón (1979) who reported most of the shallow reefs surrounding the island as dominated by *A. palmata*. A few sites described by Goenaga and Cintrón (1979) have been studied over time in Culebra, Fajardo, Guánica, Mona Island and La Parguera, yet no comprehensive monitoring directed towards this species is currently in place. In order to assess *A. palmata* population status this study examines the distribution at large spatial scales combined with localized demographic surveys to determine coral colony condition and existing threats.

Puerto Rico contains the greatest extent of Elkhorn coral in the US Caribbean. The main island of Puerto Rico has been identified as within the genetic population of the eastern Caribbean while Mona Island has been proposed as a mixing zone for the eastern and western populations for the species (Baums et al. 2005). This makes the area an important region to understand population dynamics and provide data towards evaluating recovery of the species.

The goal of this project was to determine the current distribution and condition of Elkhorn coral in Puerto Rico, to provide a basis to assess population status and trends in the archipelago. The first objective was an island wide calculation of the potential habitat for the species. Secondly, a subset of the area was sampled in order to determine the presence and condition of *A. palmata.* Finally, long-term demographic monitoring was initiated utilizing permanent plots.

Materials and Methods

A geographical information system (GIS) was used to create a spatial database in order to delineate the potential habitat of Elkhorn coral around all Puerto Rico. Potential habitat for Elkhorn coral was defined as the areas where occurrence of the species is possible, namely areas of hard substratum to 15m depth. Bathymetric and benthic habitat (NOAA 2001) layers were used to select

areas between 1 and 15m of hard-bottom habitat. These areas were stratified by depth into high probability of encountering Elkhorn in shallowest depths (0-5 m), medium probability in mid depths (5-10 m) and low probability in deeper regions (10-15 m). All map calculations were conducted at 50m cell size. Benthic habitat maps were used to extract areas of unconsolidated sediments and submerged aquatic vegetation. Some areas of habitat classified as 'unknown' due to low water clarity in the aerial photos were included if they were located in areas of suitable depths.

In order to quantify Elkhorn abundance, areas of potential habitat with a high probability of encountering Elkhorn coral were chosen within 6 marine protected areas (MPA) on the east and west coast of the island. The study focused on these MPAs because of their importance to coral reef conservation and to provide an island-wide perspective, although they may not represent all areas of potential habitat. Arrecifes de la Cordillera Natural Reserve (NR) is located off of Fajardo on the northeastern coast of Puerto Rico and includes multiple cays and reefs, while Canal Luis Peña NR in Culebra and Bahías Bioluminiscentes de Vieques NR are located along the eastern coast. Tres Palmas Marine Reserve (MR) in Rincón and Punta Guaniquilla NR in Cabo Rojo are on the main island's western coast, while Arrecifes de Tourmaline NR is part of an offshore reef system located 9.5 km west of Puerto Real, Cabo Rojo.

Within the potential habitat (high probability, <5m depth hardbottom) of each MPA randomly chosen points were selected in GIS (ArcMap 9.1 and Hawth Tools extension) and uploaded to a handheld GPS. In the field a leaded line with surface buoy was used to mark the randomly chosen point to center the $100m^2$ survey area. At each survey area the presence of *A. palmata* and dead standing skeletons within the $100m^2$ was noted by delimiting the area with a floating line (5.6m) used as radius of the circular plot. When live *A. palmata* were present the number, size (maximum length, width and height) and percent live coral cover of each colony was measured for those colonies with their center within the survey area. Colonies less than 50 cm in minimum length were not quantified in these surveys. A Live Area Index (LAI) was calculated (length*width*% live/100) for each colony as an estimate of total coral tissue. Elkhorn colony measures were compared between sites with non-parametric statistical methods. In addition, colony condition variables were assessed including bleaching, disease and predation by invertebrates.

Data on the distribution and condition of *A. palmata* were then used to establish permanent long-term demographic monitoring stations following the protocol of Williams et al. (2006). These sites have been sampled semi-annually since October 2007. All colonies within a $150m^2$ circular plot were measured, photographed, and 12 randomly chosen colonies were permanently tagged for long-term monitoring to quantify their survivorship, growth and changes in condition. At three MPA sites three circular plots (150 m^2) were established with permanent markers.

A size frequency distribution based on classes determined by amount of live tissue was used to show population structure patterns. Colonies between 60 cm^2 and 1,600 cm^2 have low-reproductive potential, while colonies below 15–60 cm^2 are not reproductively active (Soong and Lang 1992). Based on these estimates, large (presumably adult) colonies were considered to be those larger than reproductive size (1,600 cm^2), medium those below minimum reproductive size (50–1,600 cm^2), and small those not expected to reproduce (<50 cm^2). The LAI of tagged colonies per plot was compared between baseline and month 6.

Results

The island-wide potential habitat for Elkhorn coral was estimated at 673 km^2 (67,354 ha). This was classified into areas of high (16% of total area), medium (30%) and low (54%) probability of encountering the species. The area of potential habitat with the highest probability of finding Elkhorn coral was 106.5 km^2 (10,651 ha). The proportion of high probability potential habitat area in the 6 MPAs varied inversely to total MPA size (Table 1).

Table 1: Sampling areas in MPAs on the east and west coast of Puerto Rico and the percent of submerged area (Area) which is considered potential habitat (high probability) for *A. palmata* within each MPA

	Marine Protected Area	Area (ha)	% Potential Habitat
WEST	Tres Palmas (**TP**)	83	62.4
	Arrecifes Tourmaline (**AT**)	7,269	1.3
	Punta Guaniquilla (**PG**)	1,947	2.7
EAST	Arrecifes de la Cordillera (**AC**)	10,082	2.5
	Canal Luis Peña (**CLP**)	633	12.6
	Bahías Bioluminiscentes de Vieques (**BB**)	7,962	1

Elkhorn density and colony measurements were conducted at 431 randomly selected survey areas within the high probability potential habitat of 6 MPAs from December 2006 through October 2007. Overall 30.7% of all points sampled had live *A.*

palmata colonies, yet this varied from 4% at Punta Guaniquilla NR to 73% at Arrecifes de Tourmaline NR showing significant differences in the distribution of live *A. palmata* (Fig. 1). Overall 13.7% of points sampled had *A. palmata* dead standing skeletons or rubble indicating the species used to occur in these areas. This also varied greatly from 0% at Tres Palmas MR to 42% in Arrecifes de la Cordillera NR. Colonies were absent in 27.5% of all points, ranging from 13% at Arrecifes de Tourmaline to 50% at Arrecifes de la Cordillera. Areas where *A. palmata* was absent were gorgonian dominated hard-bottom or uncolonized pavement with sand.

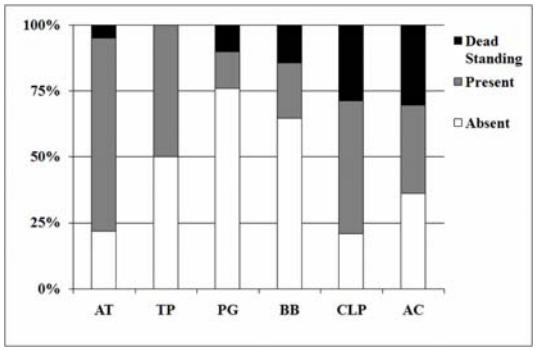

Figure 1: Presence of Elkhorn coral within the high probability potential habitat areas of: **AT** = Arrecifes de Tourmaline (N=60), **TP** = Tres Palmas (N=56), **PG** = Punta Guaniquilla (N=29), **BB** = Bahias Bioluminiscentes de Vieques (N=48), **CLP** = Canal Luis Peña (N=100) and **AC** = Arrecifes de la Cordillera (N=138).

Colony density varied greatly and ranged between 0 and 52 colonies/100m^2. The greatest abundance of *A. palmata* was observed at two west coast MPAs (9.8 colonies/100m^2 for Arrecifes Tourmaline NR and 7.1 colonies/100m^2 for Tres Palmas MR) where the overall mean was significantly higher (Kruskal-Wallis test p=0.00) (Fig 2). On average the largest colonies were observed at Tres Palmas MR and Arrecifes de Tourmaline NR while the greatest proportion of smaller sized colonies (50-99 cm length) was seen at Canal Luis Peña NR.

A total of 1,387 colonies were quantified and measured throughout all MPAs. The number of colonies assessed per study site varied from 5 at Punta Guaniquilla to 586 at Arrecifes de Tourmaline. Due to the low number of colonies available in Punta Guaniquilla NR and Bahías Bioluminiscentes de Vieques NR the data from these areas is not included in further analyses.

Percent live tissue cover per colony averaged highest at Arrecifes de la Cordillera NR (87%) followed by Tres Palmas MR (77%) and was lowest at Canal Luis Peña NR (61%). Mean LAI varied between 1.4 and 14.9 m^2 per survey area. Colonies at west coast sites showed significantly higher LAI (Kruskal-Wallis test p=0.00) over east coast sites (Fig 3).

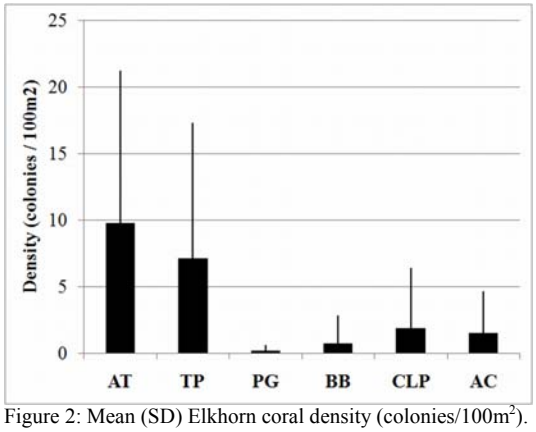

Figure 2: Mean (SD) Elkhorn coral density (colonies/100m^2).

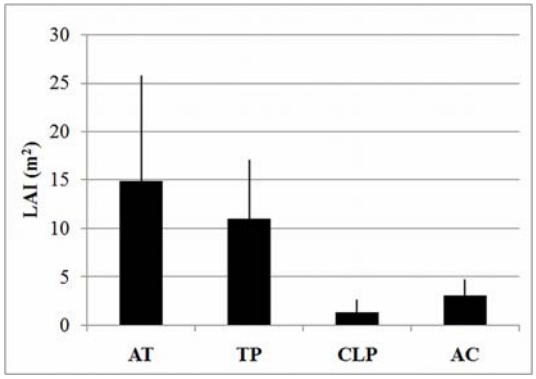

Figure 3: Mean (SE) live area index (LAI) per survey area (m^2) for survey areas where *A.palmata* was present.

Infestations of corallivorous snails (*Coralliophila abbreviata*) were observed on 3% of all colonies although this ranged from 0.9% in Arrecifes de Tourmaline NR to 10.6% at Canal Luis Peña NR. Snail occurrence rates were higher on the east coast which had lower Elkhorn densities and smaller colonies. Average number of snails per colony ranged from 2.6 for Arrecifes de Tourmaline NR to 5.1 at Arrecifes de la Cordillera NR. The frequency and number of snails was highest at Canal Luis Peña NR and Arrecifes de la Cordillera NR and the occurrence was inversely related to the density of colonies over all sites sampled.

Disease was observed on 6.7% of colonies averaged over all sites (range from 4% at Tres Palmas MR to 9% at Arrecifes de Tourmaline NR) and most of the diseased colonies were classified as such due to patchy necrosis (white pox disease). White band disease (WBD) affected 2.4% of all colonies and was most prevalent in Arrecifes de la

Cordillera NR (4.7%) and Canal Luis Peña NR (1.6%) while only 1% occurred at Arecifes Tourmaline NR and none were observed at Tres Palmas MR. Partial bleaching (<20% of colony) was observed in Tres Palmas MR (3 colonies) and Arrecifes de Tourmaline NR (2 colonies) on the west coast. Recent mortality estimates ranged from 0.1% of the colony to 40%. The greatest percentages of the colony affected by recent mortality were attributed to competition with the encrusting sponge *Cliona tenuis* (only at west coast sites) and diseases.

Those MPAs with lower densities of live *A. palmata* (Punta Guaniquilla NR, Canal Luis Peña NR and Bahías Bioluminiscentes de Vieques NR) were not considered suitable for permanent long-term monitoring. This was mainly due to the limitations of the protocol for demographic monitoring (Williams *et. al*. 2006) in which areas with low density of *A. palmata* or depths less than 2 m are unsuitable. Three permanent monitoring plots were established at each of three MPAs (Arrecifes Tourmaline NR, Arrecifes de la Cordillera NR and Tres Palmas MR) in October of 2007 for demographic purposes. The frequency distribution of size classes in the permanent plots showed a greater proportion of small colonies in Arrecifes de la Cordillera (Fig. 4).

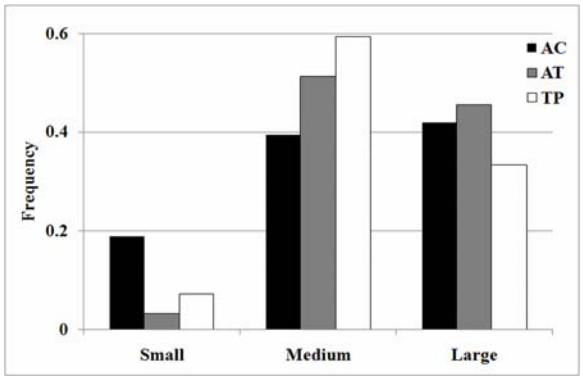

Figure 4: Frequency distribution of all colonies by size classes at long-term monitoring plots in AC (N= 117), AT (N=156) and TP (N=138). Size classes based on amount of live tissue: Small < 50 cm^2, Medium 50 cm^2 – 1,600 cm^2, Large > 1,600 cm^2.

After six months, three (2.7%) permanently tagged colonies were dead and 18 (16.7%) were unaccounted for within the permanent plots. No significant changes in live tissue cover were observed for the colonies that remained. The mean LAI for each plot decreased at Arrecifes de la Cordillera over the first six months of sampling (Fig. 5).

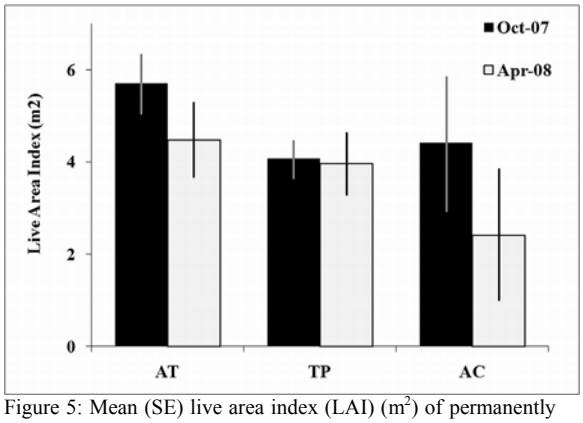

Figure 5: Mean (SE) live area index (LAI) (m^2) of permanently tagged colonies per plot over two sampling periods.

Discussion

In this study we estimate that potential Elkhorn coral habitat occurs in an area approximately 673 km^2 around the Puerto Rican archipelago. In order to improve this estimate greater resolution bathymetric and habitat maps are necessary to refine the scale of potential habitat. Ground-truthing of additional sites where it was documented in the past (Goenaga and Cintrón 1979) will help improve the estimate further accounting for spatial variability as this species is known to be patchy in distribution.

Within the 6 MPAs the proportion of area where Elkhorn coral was present (30% of high probability potential habitat) was lower than an island-wide estimate of 88% conducted during the late 1970's (Weil et al*.,* 2002), although methods differ. On the eastern coast the presence of Elkhorn was observed at 37% of sites (Weil et al*.,* 2002) which is comparable to this study's estimate of 36% pooled for three east coast sites. Nonetheless, total loss in Elkhorn coral was evidenced in 13.6% of the random survey areas where only dead standing skeletons were present (range 0% - 42%) which can be extrapolated to an estimate of 15 km^2 island-wide and is supported by the observation of large thickets of dead standing Elkhorn.

The proportion of random survey areas where live Elkhorn was present varied greatly among MPAs (14% - 73%) and the higher estimate from Arrecifes Tourmaline is comparable to results obtained by Mayor et al. (2006) (74%) in St. Croix, USVI. However, the maximum depth sampled in Puerto Rico was 5 m and in St. Croix it was 10 m. The overall density from this study (3.3 colonies/100 m^2) was higher than St. Croix (1.6) and Venezuela (3.1) (Mayor et al*.,* 2006; Zubillaga et al*.,* 2007), however comparison of density estimates among studies is troublesome due to difference in methods and the limits of areas surveyed.

On average the Elkhorn populations within the MPAs on the east coast of Puerto Rico seem to be in relatively poorer condition due to lower density, size, live coral cover and higher rates of disease, recent mortality and predation by snails. Canal Luis Peña, Culebra had the lowest mean live coral cover per colony and the smallest sized colonies with resulting low mean LAI. Arrecifes de la Cordillera had the highest mean live tissue cover per colony of all sites but it lacked large sized colonies which reduced the mean LAI for the site. Since small colonies make up a greater proportion of the population at east coast sites this may reduce their ability to withstand bleaching, disease and predation impacts as less live tissue is available to be lost.

White band disease (WBD) was observed in 2.4% of all colonies yet the greatest proportions were observed in east coast sites of Puerto Rico. In St. Croix the overall prevalence of WBD was 3.2% (Mayor *et. al.,* 2006) while in Los Roques, Venezuela it varied between 0.4 and 4.7% (Zubillaga *et. al.,* 2007) suggesting that Elkhorn coral threats demonstrate high variability within geographical regions, although temporal factors may also affect the patterns observed.

The overall proportion of colonies with snails averaged 3.7% (range 0.9 to 10.6%) which is lower than previous reports for Puerto Rico (e.g., 18%, Bruckner *et. al.,* 1997), yet this is highly variable. At east coast sites colonies had groups of 10 to 18 snails while the west coast sites had occurrences of less than 6 snails per colony. Mean number of snails per colony was 5.1 at Arrecifes de la Cordillera, higher than the 3.7 estimated for La Parguera (Bruckner 2000).

Long-term monitoring for Elkhorn coral in Puerto Rico initiated in 2007 showed a decrease in LAI for colonies tagged in Arrecifes de la Cordillera. Although no significant changes in mean percentage of live tissue cover were detected, the loss of 12 colonies at this site reduced total plot LAI. The loss of whole colonies was caused by the unusually large swell event that occurred during March of 2008 with long period waves reaching up to 12 m in height (National Weather Service, NOAA). Other sites also had detached and broken colonies, yet impact was most notable in northern sites of Arrecifes de la Cordillera and at Tres Palmas MR the number of loose fragments was lower than at other sites.

The potential habitat for *A. palmata* calculated for the Puerto Rican archipelago is a first step towards better understanding the population's spatial and temporal trends. As a population declines, a combination of reduced extent and density is expected, yet extrapolating results from a few sites can be misleading. Our results suggest differences in the occurrence, distribution and condition over the scale of Puerto Rico. The method used to evaluate the distribution of Elkhorn within MPAs provides a spatially explicit baseline upon which changes can be evaluated, which in combination with the demographic monitoring will enhance our understanding of population dynamics of Elkhorn coral in the Puerto Rican archipelago.

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The prevalence of skeletal growth anomaly and other afflictions in scleractinian corals at Waiʻōpae, Hawaiʻi

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Abstract. The health states of scleractinian corals were assessed at Waiʻōpae, southeast Hawaiʻi Island, where approximately half of the tide pools belong to a Marine Life Conservation District (MLCD) while the other half lies outside the MLCD boundary. The susceptibility of corals to afflictions is species-dependant with *Porites compressa* being the most (22.5±3.9% unhealthy) and *Pavona varians* the least (1.1±0.27% unhealthy) susceptible. The proportion of unhealthy corals was: higher inside the MLCD than outside for *Montipora* and *Pocillopora*; not different between the two areas for *Porites*; and higher outside MLCD than inside for *Pavona*. Skeletal growth anomaly (SGA) was disproportionably observed in *Montipora* spp., especially *M. capitata*, 13.6 (±0.37) % of which were afflicted by this condition. There is no clear correlation between the progression of SGA (change in size or frequency) and species, location, or season. Some coral colonies are showing a level of resilience against SGA while others have succumbed to it within the Waiʻōpae population. The prevalence of coral afflictions at Waiʻōpae is unusually high compared to other sites in the Hawaiian Archipelago, but the causes are unknown. Monitoring of coral health at this site is continuing with an incorporation of assessment of correlations to water quality characteristics.

Key words: coral disease, skeletal growth anomaly, Waiʻōpae, Hawaiʻi, *Montipora*

Introduction

Prevalence and severity of coral diseases are reported to be increasing worldwide (reviewed in Harvell et al. 2007). Types of diseases or reduced health states seem to vary according to coral species and location. In many cases, the effort to positively identify the pathogen responsible for the onset of coral diseases has been met with challenges. It is generally believed that water quality exacerbates the susceptibility of corals to pathogenically or non-pathogenically compromised health. For example, moderate increases in nutrient concentrations have been shown to substantially increase the severity of coral diseases (Bruno et al. 2003). However, specific causal relationships between particular water quality characteristics and individual coral disease are poorly understood. With the mechanisms of disease progress and mortality of corals being poorly understood, it is quite plausible that disease could even enable coral survival through reproduction of disease resistant genotypes in some cases (Lesser et al. 2007). Until the effects of coral diseases are better understood it is difficult to assess the fate and threat posed to corals in waters subjected to harmful anthropogenic influences.

Coral reefs surrounding high gradient island systems, such as Hawaiʻi, are particularly vulnerable to influences from terrigenous sources. This is especially true on the island of Hawaiʻi because the substrate is predominately composed of porous basaltic rock and, being young on a geological timescale, lacks an abundance of soil for bioremediation. Further compounding these effects is the human population growth. Hawaiʻi Island has experienced one of the fastest human population growths over the last decade or so (23.6% increase in 1990-2000) in the State of Hawaiʻi (U.S. Census Bureau 2000). Synergistically, these factors have a great potential to produce direct anthropogenic effects on fringing coral reef ecosystems.

Some of these effects can be manifested in states of reduced health of corals. Several symptoms for poor health in reef-building corals have been reported in Hawaiʻi and range from bleaching, coral tissue loss, infectious diseases for which pathogens have been identified, and other abnormalities in cellular, tissue, and skeletal morphologies of unknown causes (Gulko et al. 2000, Friedlander et al. 2005, Sutherland et al. 2004). Coral diseases have been found on most major coral species of the main Hawaiian islands (*Porities lobata, P. compressa, Montipora capitata, M. patula, and Pocillopora meandrina*: Gulko et al. 2000, Friedlander et al. 2005). The last few decades has seen an increase in the frequency of coral diseases over a range of depths and habitats in Hawaiʻi (Gulko et al. 2000, Friedlander et. al 2005). Different coral genera exhibit different types and prevalence of diseases at different locations in Hawaiʻi (Aeby 2004, this study). These conditions, with the exception of

bleaching, are poorly studied. In fact, it is not clear how well these coral conditions adhere to the definition of disease because the mechanisms and degree of functional interruption are largely unknown.

Hawai‘i Island is the largest of all Main Hawaiian Islands and contains some of the most well-developed coral reefs. However, data on diversity, abundance, and distribution of compromised health conditions in corals around Hawai‘i Island are largely absent thus far. Our monitoring program has been surveying corals in the tide pools at Wai‘ōpae, Hawai‘i Island, the southern part of which was designated as a Marine Life Conservation District (MLCD) where no collection of any organisms is allowed. The initial results of prevalence rates and changes in types of unhealthy states in corals from 2006 to 2008 are presented here.

Material and Methods

All field surveys were conducted in Wai‘ōpae tide pools that fall inside and outside of the MLCD (19°29’55”N 154°49’06”W, Fig. 1).

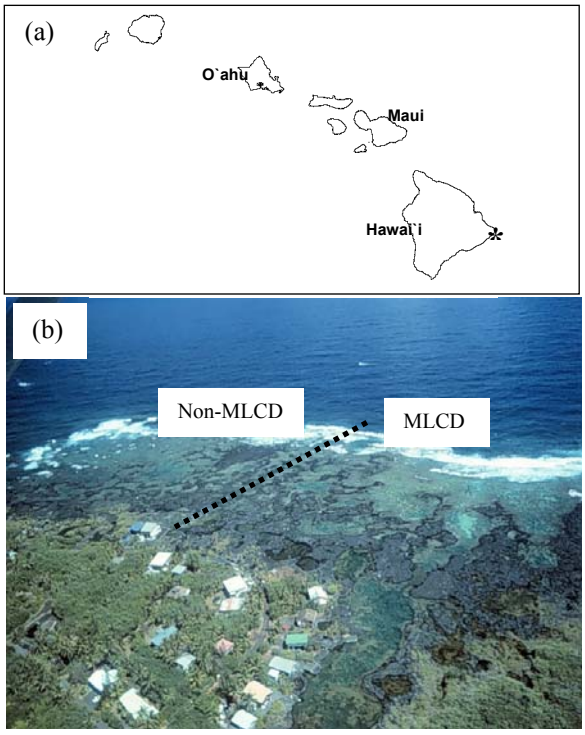

Figure 1: (a) Map of Main Hawaiian Islands showing the location of Wai‘ōpae on Hawai‘i Is., indicated by *. (b) Aerial photograph of Wai‘ōpae tide pools showing the boundary (broken line) of the Marine Life Conservation District. Note that this photograph was taken circa 1999. Currently there are many more houses along the shoreline. Photo taken by J. Coney, UHH.

For transect surveys, coral colonies along six randomly placed 25m transect lines each inside and outside of the MLCD were surveyed on four occasions in 2006 and again in June 2008. Coral colonies along these transects were identified to species, and proportions of colony areas occupied by various health states were visually estimated. To monitor changes in the health state of individual coral colonies, locations of four colonies of each of the six most common coral species(*Montipora capitata, M. flabellata, M. patula, Porites lobata, Pocillpora meandrina, Pavona varians*) were recorded inside of the MLCD, and four additional colonies of each species outside of the MLCD. These coral colonies were originally randomly selected regardless of their health status and repeatedly observed monthly between Feb 2007-March 2008 by both visual inspection and image analysis. For the image analysis, a photo was taken from the plain parallel to the coral colony within the frame size that fit the entire colony. The relative areas of the entire colony and that of unhealthy states were calculated using an image analysis software, ERDAS Imagine 9.1 (Leica Geosystems) at the GIS Laboratory at University of Hawai‘i at Hilo. All statistical analyses were performed using Minitab 15 (Minitab Inc.).

Results

The pooled survey data from randomly placed transects in 2006 and 2008 showed that the ten most common scleractinian coral species found at Wai‘ōpae had significantly different (Kruskal-Wallis test, $p<0.001$) prevalence of colonies showing symptoms of any unhealthy states, with *Porites compressa* and *Montipora capitata* being most unhealthy and *Pavona varians* being the most healthy (Fig. 2).

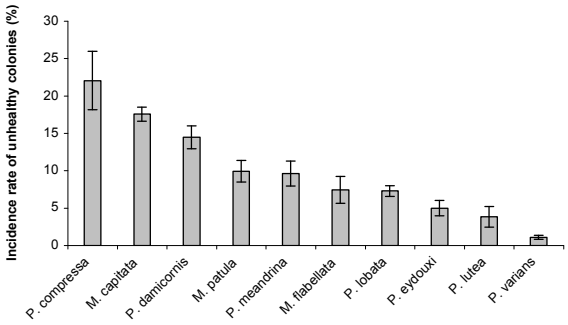

Figure 2: Mean incidence rates (% ± std. error, n=6-694) of unhealthy corals according to species at Wai`ōpae, Hawai‘i pooled from the 2006 – 2008 random transects.

The susceptibilities to different types of afflictions were species- and genus-dependent. Comparison among common genera showed significant differences among genera in susceptibility to bleaching, skeletal growth anomalies, and tissue loss of unknown causes (Kruskal-Wallis, $p<0.001$) with

Montipora being the most susceptible to all three conditions (Fig. 3).

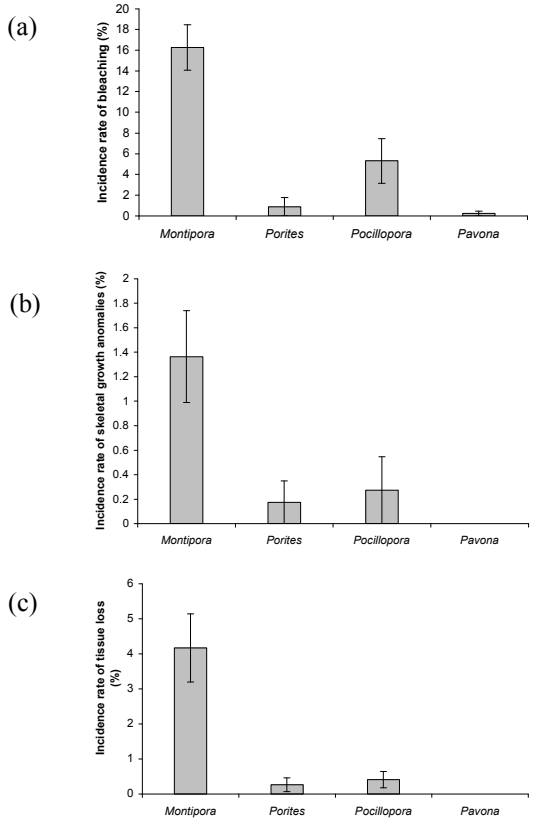

Figure 3: Mean incidence rates (% ±std. error, n=22-121) of (a) bleaching; (b) skeletal growth anomalies; and (c) tissue loss observed in the four most common coral genera at Wai`ōpae, Hawai'i from the 2006 – 2008 random transects.

Comparisons of incidence rates of unhealthy colonies between inside and outside of the Wai'ōpae MLCD revealed no consistent trend across coral genera (Fig. 4). However, the individual genus data showed that the prevalence of unhealthy colonies was higher inside the MLCD than outside for *Montipora* and *Pocillopora* (both $p<0.00$), same between two areas for Porites ($p>0.05$), and lower inside the MLCD than outside for *Pavona* ($p<0.05$) (Fig. 4).

The monthly repeated observations of coral colonies representing the six most common species have produced a large database of image archives that will continue to expand. With these images, skeletal growth anomalies were the easiest to track since the growth anomalies have well defined boarders that can be traced by image analysis. *Montipora capitata* showed the highest incidence rate of skeletal growth anomalies of all coral species in the random transect surveys of 2006-2008 (19.32±3.91%, data not shown).

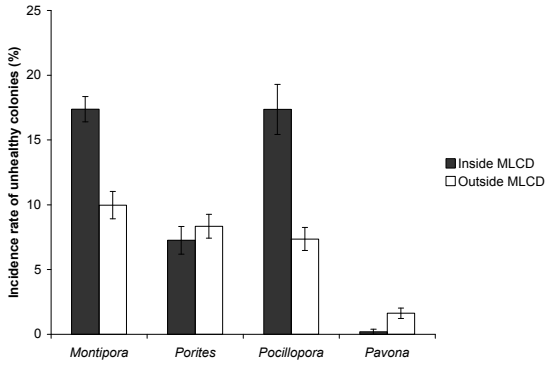

Figure 4: Comparison of the mean incidence rates (% ± std. error, n=77-612) of unhealthy corals inside and outside of the Wai'ōpae Marine Life Conservation District (MLCD) separated into the four main genera.

The size of skeletal growth anomalies was expressed as relative to the entire coral colonies that housed them to account for growth of corals. Generally, the relative size of skeletal growth anomalies increased slightly or remained the same both inside and outside of the MLCD between February 2007 and February 2008 (Fig. 5). In one of the 8 colonies, the skeletal growth anomaly occupied >90% of the entire colony surface for the duration of our observations, eventually resulting in death of the entire colony by February 2008 (Colony "In 1", Fig. 6a-b). Other *M. capitata* colonies maintained the same relative sizes of skeletal growth anomalies (e.g. Colony "In4", Fig. 6c-d) or remained completely devoid of growth anomalies for the same observation period. Overall, there were no significant correlations between the skeletal growth anomalies (change in size or frequency) and species, location, or season (all $p>0.05$).

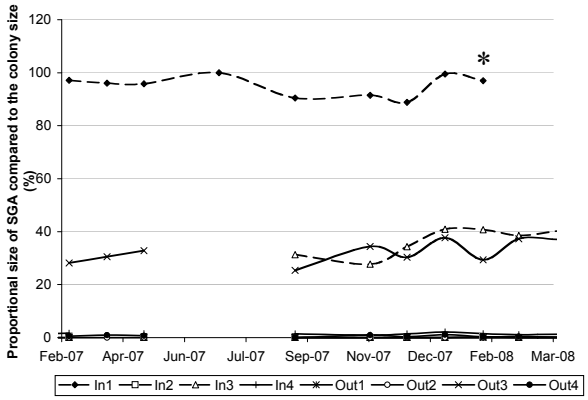

Figure 5: Changes in the sizes of skeletal growth anomaly (SGA) proportional (%) to the whole colony size of four repeatedly analyzed *M. capitata* colonies inside the MLCD ("In1"-"In4") and four colonies outside ("Out1"-"Out4"). Data for July 2007 for colonies except Colony "In1" are unavailable. *The first colony inside the MLCD ("In1") completely died; see Fig. 6.

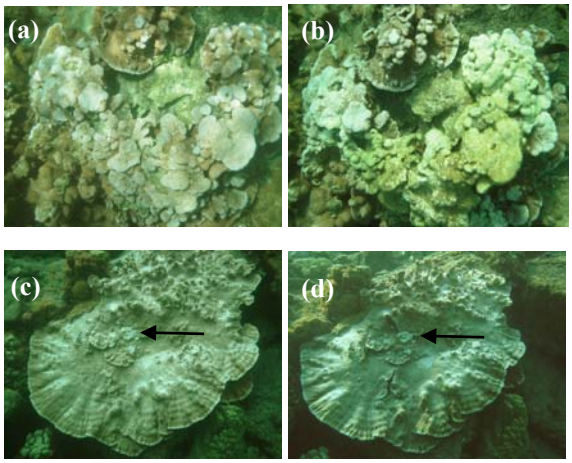

Figure 6: Examples of changes in skeletal growth anomalies in repeatedly observed *Montipora capitata* colonies at Wai`ōpae, Hawai'i. Colony "In1" in February 2007 (a) and in February 2008 (b), showing the expansion of skeletal growth anomalies (shown as white patches with anomalous textures in the photos) resulting in the colony's death by February 2008. Colony "In4" in February 2007 (c) and in February 2008 (d), showing no change in the relative size of the skeletal growth anomaly (indicated by arrows) over the same period of time.

Discussion

Hawaiian Archipelago showcases geologic history in a linear geographic scale from the southeast end to the northwest extreme. Hawai'i is the southern-most, and thus youngest (<500,000 years old, Juvik and Juvik, 1998), island of the archipelago. The incipient fringing reefs around Hawai'i Island, therefore, signifies the beginning of coral reef ecological succession along the Hawaiian island chain. Despite such a young geologic age, the reefs at Wai'ōpae on the east side of Hawai'i Island boast a relatively high level of live coral cover (mean 38.9% cover outside of the MLCD and 44% inside, 2004 surveys Hallacher et al. unpublished). Our monitoring program that began in June 2006 which is still continuing has depicted the health status of coral species there.

The incidence of unhealthy corals at Wai'ōpae is much higher than other Hawaiian locations. What is especially anomalous, visibly and statistically, is the number of *Montipora* that have succumbed to afflictions, especially to skeletal growth anomalies. For example, Aeby (2004) found the mean incidence rate of unhealthy *Montipora* to be less than <1% in uninhabited Northwestern Hawaiian islands, compared to 22.1% at Wai`ōpae. Our preliminary surveys have found that the proportions of *Montipora* colonies with skeletal growth anomaly were <1% at Leleiwi and Ke`ei (data not shown), both sites near relatively high human populations on Hawai'i Island.

There is an on-going debate on whether pathogens are the primary cause of coral diseases or if pathogens incite secondary opportunistic infection or reaction when corals are already under physiological stress (Harvell et al. 2007, Lesser et al. 2007). The physiological function of coral colonies may change due to environmental stress affecting the coral, resulting in altered environmental conditions for coral-associated bacteria (Pantos et al. 2003). Regardless of directness of the link, reports are suggesting that increases in the number of both new diseases and species affected are linked to human-induced alterations in coral reef environments both in terms of land-based sources of pollution, sedimentation, and eutrophication as well as global climate changes such as seawater temperature rises and ocean acidification (Bruckner & Bruckner 1997, Harvell et al. 1999, Porter et al. 2001, Rosenberg & Ben-Haim 2002, Sutherland et al. 2004, Bruno et al. 2003, Pantos et al. 2003, Aeby 2004). Most studies have been unable to identify exogenous organisms or intracellular infection by parasitic agents for skeletal growth anomalies. It has been suggested that anomalous skeleton formation and growth may be influenced by environmental factors; however, no clear correlation has been established. Several investigators have postulated that disease incidence might be associated with elevated nutrients with or without sediment loads delivering potentially pathogenic organisms to the marine environment (Richardson 1998, Sutherland et al. 2004, Breitbart et al. 2005, Bruno et al. 2003).

Causes of the observed high rates of unhealthy states in corals at Wai'ōpae are also not known. The porous basaltic substrate and proximity of the reef to the coastline due to the absence of continental shelf or well-developed reef crest found at this site are typical of other windward locations on the same island. What might be negatively affecting environmental conditions for benthic organisms at Wai'ōpae is the reduced water circulation due to the substrate forming tide pools. All of the corals that were surveyed in this study were found in tide pools with varying levels of connection to open water. The water circulation pattern in Wai'ōpae tide pools has not been investigated but is expected to be very complex because of the high level of complexity in substrate topography as well as freshwater inputs through groundwater and land runoffs. At low tides, many of the tide pools are ponded, whereas the seawater level at high spring tides covers the tops of tide pool walls and reaches the residential houses along the shoreline.

Water quality inside the tide pools would likely affect benthic organisms such as corals more than mobile organisms. If coral afflictions at Wai'ōpae are direct or indirect results of terrigenous stressors, than accumulation of runoff, groundwater, and reduced water circulation at low tides must be exacerbating their effects. This might partially explain why the

coral genera that are more susceptible than others, such as *Montipora* and *Pocillopora*, are showing higher unhealthy rates inside the MLCD than outside the MLCD (Fig. 4). The tide pools inside the MLCD are more isolated and ponded at low tide than those outside the MLCD (Fig. 1b). Another compounding effect that has the potential of creating differences in stressor level in tide pools inside and outside the MLCD is the human use. Of the estimated 87,190 people who visited Waiʻōpae during 2004-2005, 90.0% of them stayed within the 50-acre MLCD (Shea Flanders and Settlemier, 2007). A larger proportion (36%) of people participated in non-consumptive underwater activities, such as snorkeling, inside the MLCD than outside (21%, Shea Flanders and Settlemier, 2007). The MLCD at Waiʻōpae was designated as a no take zone in 2003. The effect of this MLCD designation needs to be carefully assessed in terms of the benefits of various species protection at the cost of increased visitor attraction and usage. These results from coral monitoring illustrate the complexity of the many factors influencing coral health. More detailed analysis comparing multiple environmental differences inside and outside the MLCD may shed light on what elements must be incorporated into conservation protection in order to adequately address coral health concerns.

It is not known when the health states of corals at Waiʻōpae began to deteriorate to the present levels because there had been no regular monitoring effort focusing on the health of corals there before this program. Regarding skeletal growth anomalies specifically, the disease state does not seem to advance rapidly (Fig. 5 and Fig. 6). One exception to this was found in the colony, "In1", that was heavily (>90%) covered with skeletal growth anomaly for 10 months of our observation without showing further deterioration before succumbing rapidly to turf algal invasion in the last two months (Fig. 5). In order to monitor changes in health conditions of corals at Waiʻōpae effectively, we will continue our periodic random transect surveys and photo documentation of repeatedly observed colonies. Current efforts are being focused on evaluating the water quality in Waiʻōpae tide pools and assessing potential correlations between water quality characteristics to incidence, types, and progression of coral afflictions.

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The Keaholoa Native Hawaiian STEM Internship Program (NSF, T-CUP, PI S. Juvik), the Louis Stokes Alliances for Minority Participation (NSF, LSAMP, PI R. Tseng), and University of Hawaiʻi at Hilo Seed Money grant to M.T. provided funding for this project, for which authors are grateful. We also thank the field assistance from S.K. Tom, M. Manuel, N. Olson, N. Goo, J. Moriarty, and E. Murphy.

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Disturbance and recovery of mangrove forests and macrobenthic communities in Andaman Sea, Thailand following the Indian Ocean Tsunami

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Abstract. On December 26, 2004, the impact of tsunami waves caused by an undersea earthquake off Sumatra Island generated severe damage to coastal communities throughout the Indian Ocean. One year before the Indian Ocean Tsunami (IOT), we established permanent survey plots in characteristic mangrove forests on the coast of Ranong, Thailand. Since then, we have continued to survey mangrove stands and macrobenthic communities. Damage caused by the IOT included immediate damage and gradual indirect mortality, but the damage to mangrove ecosystem was limited and habitat specific with 6% to 16 % of mangrove stands showing damage one year after the tsunami. Although population density and biomass of macrobenthic organisms were not affected by the tsunami, diversity decreased in some areas. The community structure of macrobenthic organisms in mangrove swamps was affected: endobenthic organisms decreased due to an increase in the abundance of coastal sand in the sediments; epibenthic organisms initially decreased but recovered gradually; and terrestrial epibenthic organisms increased on dried sandy substratum.

Key words: natural disaster, Thailand, tsunami, macrobenthos, Andaman Sea, mangrove forests

Introduction

Mangroves play important roles in coastal ecosystems of the tropics and sub-tropics producing of a lot of litter which provides food and energy to the aquatic flora and fauna. Mangroves also provide fundamental ecological services to local inhabitants (Aksornkoae 1993; Hogarth 1999; Barbier and Sathirathai 2004). Many studies have been done on mangrove distribution and productivity, both in forest stands and aquatic systems, however, there is insufficient knowledge on the disturbance of mangrove ecosystems by large natural disasters such as the tsunami which is different from usual ocean waves and on the process of recovery from such catastrophic damage.

On December 26th, 2004, the impact of tsunami waves caused by an undersea earthquake off Sumatra Island generated severe damage to artificial constructions and some natural ecosystems on coastal area along the Andaman Sea (MNRE 2005; UNEP 2005; Stoddart 2007). Mangrove stands suffered unprecedented damage by both direct impact from the waves and floating material and also more gradual indirect symptoms caused by root damage due to piles of thick sand brought by the tsunami (Matsumoto et al. 2006). The tsunami also disturbed fish and benthic communities which play a very important role in mangrove ecosystems.

One year before the tsunami disaster, we established permanent survey plots in some characteristic mangrove forests on the coast of Ranong area facing the Andaman Sea, Thailand. Since then, we monitored regularly the dynamics of mangrove forests and macrobenthic communities which utilize them as habitat and potential food sources. These areas suffered severe damage from the tsunami on human lives, infrastructure and mangrove ecosystems, and therefore, we could obtain comparable data to show disturbance patterns and to evaluate the recovery process in mangrove ecosystems. In this study, we demonstrate the initial process of recovery from damage over a few years on mangrove forests and macrobenthic communities.

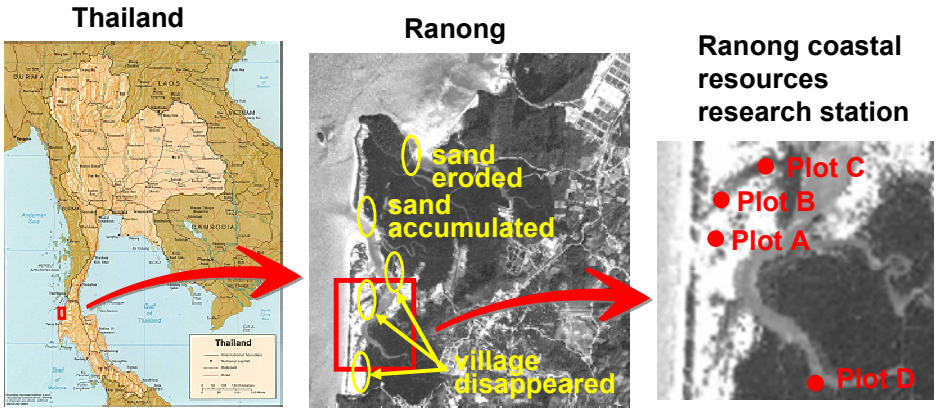

Figure 1: Map of Ranong, southern Thailand, showing the study sites in Ranong coastal resources research station, Kasetsart University

Material and Methods

Four study plots (Plots A-D) of mangrove forests were established in the vicinity of Ranong coastal resources research station (RCRRS) (9°23'N 98°24'E), Kasetsart University, situated on the Andaman coast of Suksamran sub-district, Ranong province, southern Thailand (Fig. 1). Plot A was *Avicennia alba* Blume forest located at the interior tidal inlet branched from main river. Plot B was a complex forest of *A. alba* and *Rhizophora mucronata* Lamarck located at the mouth of a tidal inlet branching off the main river. Plot C was *R. mucronata* forest on the bank of the main river. Plot D was also *R. mucronata* forest in an interior mangrove estuary outside the station. These mangrove stands were harvested and regenerated about 15-20 years previous to the begin of our study in 2003.

All mangrove trees within 500-1,000 m^2 areas were continuously monitored in every plot before and after the tsunami. Diameters above the neck of the highest root were measured. Damage to mangrove stands was estimated from loss of trees by comparing data before and the after the tsunami.

Soil and sediment at each study site was collected from the surface to a depth of 20 cm using an acrylic core with a 44 mm diameter, and grain size composition was analyzed by a series of phi-scaled mesh sieves. Taxonomic composition, density and biomass of epibenthic organisms were investigated quantitatively using a 50 cm x 50 cm quadrat set at random on the ground. Five replicate samples of sediments were collected from the surface to the depth of 10 cm at every site. The collected soils were sieved using a 500 µm mesh sieve and preserved with 10% neutralized formalin. Macrobenthic specimens were identified to the lowest taxonomic category possible. The total weight of each species was measured using an electric balance after being picked out from the residue under a dissecting stereo microscope. The survey was conducted before the tsunami on September 26, 2003 and after the tsunami on March 15, 2005 and November 23, 2006.

Population density and biomass were calculated as the number of individuals (n/m^2) and the wet weight (g/m^2) per square meter, respectively, for every species/taxa. Species richness and diversity index

Figure 2: Serious tsunami damages in the survey areas just after the tsunami (March 2005).

1. *Rhizophora* forest was destroyed by 10 m width along river (near Plot C).
2. *Rhizophora* - *Xylocarpus* stands were mown down and buried by sea sand more than 1 m thick (river mouth).
3. Concrete boardwalk for mangrove observation on *Avicennia* forest was broken by direct wave impact (Plot A)
4. Sands were carried by waves from the beach onto the floor of *Rhizophora* forest (Plot B).

were calculated for every plot. Species diversity was calculated using the Shannon-Weaver Index H'=-Σ(n/N)log(n/N), using logarithmic base 2, where 'n' is the number of individuals for each species/taxa, and 'N' is the total number of individuals.

Results and Discussion

The tsunami damaged many human lives and much property in coastal areas as well as natural ecosystems (MNRE 2005; UNEP 2005; Stoddart 2007). Examples of tsunami damages in the survey areas are presented in Fig. 2. The damage by the tsunami was caused by immediate direct impacts of floating materials as well as extremely strong wave action and backwash. Some mangrove forests suffered decline/death after being buried by sand.

In Plots A, B and C, 6.5-16.1% of mangrove stands were damaged (Fig. 3). Smaller trees suffered more damage than the larger ones, especially in Plot A (Fig. 3).

It was assumed that the damage to mangrove stands in the survey areas was due to direct and indirect causes. Gradual indirect lethal symptoms seemed to be caused by: 1) root damage due to strong shaking or disturbance of root respiration by accumulated sand carried by the tsunami; and 2) damage by sea water abnormally accumulated in the forests and the surrounding soil (Matsumoto et al. 2006).

Composition of sediment grain size of four survey plots and the neighboring beach were compared (Fig. 4). Mean particle size of sediment was the largest and well sorted in Plot C, followed in Plots A and B, and the smallest in Plot D. A considerable amount of accumulated sand (mainly 125 - 250 µm) of which the tsunami brought from the sub-tidal parts of the neighboring coast were included in the sediments of Plots A, B and C.

When we surveyed three months after the tsunami in March 2005, we found that approximately 5-10 cm of sand had accumulated on the substratum in Plots A, B and C. (observations in March 2005; Fig. 2.4). Sediment conditions recovered gradually in the two years after the tsunami. Situated in a tidal inlet the bottom substrate was exchanged frequently by routine tidal flow and by bioturbation by benthic organisms such as crabs, *Uca* spp., horn snails, *Cerithidea cingulata*, and some polychaetes.

Mollusca (36 taxa) constituteed the most dominant and diverse taxa, followed by Arthropoda (21 taxa) and Annelida (9 taxa). Dominant in the survey areas were: *Nerita violacea*, *Cerithium coralium*, *Cerithidea cingulata*, *C. quadrata*, *C. obtusa*, *Telescopium telescopium*, *Littoraria pallescens*, *L. strigata*, *Assiminea brevicula*, *Stenothyra ovalis*, *Cassidula nucleus*, *Ellobium aurisjudae* (gastropods), *Saccostrea forskali*, *Gelonia erosa* (bivalves), *Perinereis* spp. and Capitellidae (polychaetes), *Parasesarma pictum*, *Episesarma* spp., *Uca annulipes*, *U. vocans*, *Thalassina anomala* (crustaceans) and some insects.

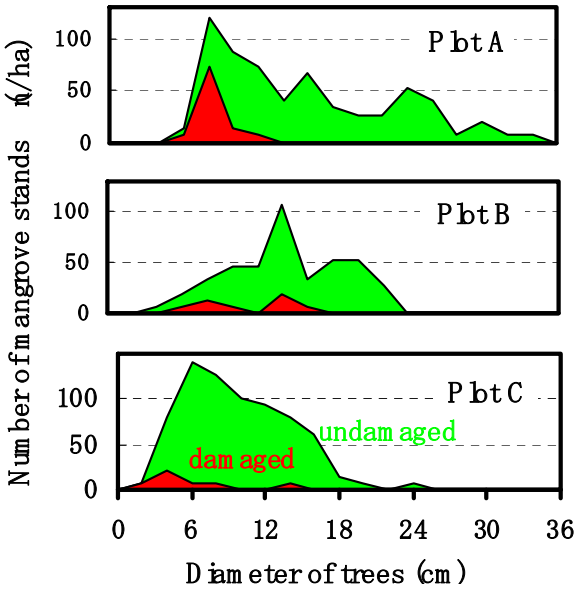

Figure 3: Damage to mangrove stands seen one year after the tsunami (until November 2005). Red: Damaged tree, Green: Undamaged tree.

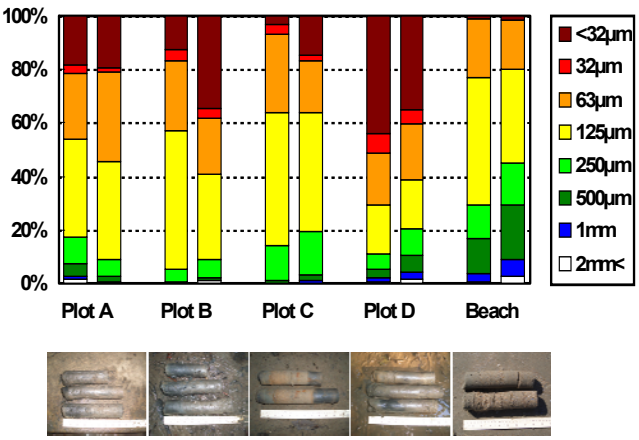

Figure 4: Composition of sediment grain size two years after the tsunami (November 2006) on four survey plots and the neighboring beach. Left bar: 0-10 cm deep, right bar: 10-20 cm deep. Photos below every plot represent the core samples in which left side of every photo is the surface layer of the substratum.

When we surveyed three months after the tsunami in March 2005, we found that almost all macrobenthic organisms, except for a few epibenthic gastropods inhabiting stem and root of mangrove trees, were buried in sand transported from nearby shallow ocean waters or the neighboring beach (Fig. 2.4). Population density (number of individuals), biomass (wet weight), and diversity (number of species/taxa and diversity indices) of macrobenthic organisms were compared before and two years after the tsunami (Fig. 5). The population density and the biomass measures were very high in Plot A in which the maximum density and biomass of horn snails, *Cerithidea cingulata*, exceeded 1,000 individuals/m^2 and 200 g/m^2, respectively, including many juveniles. In Plot D, the population density was the highest but the biomass was not large because a large number of small

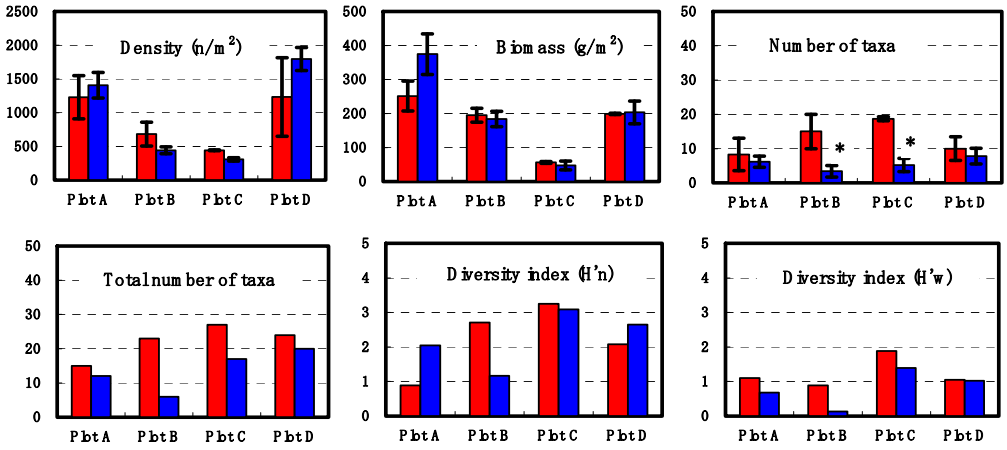

Figure 5: Community structure of macrobenthic organisms in four mangrove plots.
Red bar: before the tsunami, blue bar: after the tsunami. *mark: significant difference p<0.05.

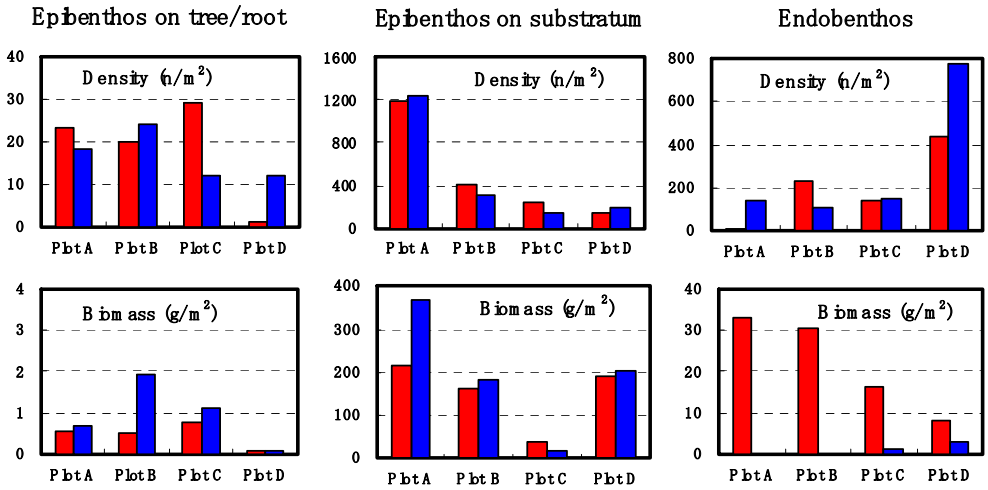

Figure 6: Population density and biomass of benthic organisms based on differences in habitat preference. Red bar: before the tsunami, blue bar: after the tsunami.

polychaetes and nematodes were included. Number of species/taxa decreased significantly after the tsunami in Plots B and C (t-test: $t = 3.89$ $p < 0.05$, $t = 14.6$ $p < 0.01$, respectively). In Plot B, the total number of species/taxa decreased 77% and the diversity index based on the number decreased 86% after the tsunami.

Population density and biomass of the following macrobenthic organisms were compared before and two years after the tsunami (Fig. 6): epibenthic organisms inhabiting stem and root of mangrove trees; epibenthic organisms inhabiting the forest floor; and endobenthic organisms living in the substratum. Although there was no distinct difference in the

density and the biomass of epibenthic organisms before and after the tsunami, the taxonomic composition changed substantially. Epibenthic organisms such as gastropods and crustaceans decreased initially (observations in March 2005) but recovered gradually. Terrestrial epibenthic organisms increased on dried sandy substratum due to immigration from neighboring forests and beaches by insects such as ants and chironomids. Biomass of endobenthic organisms decreased drastically because bivalves such as *Gelonia erosa* and polychaetes died due to the increased sedimentation by coastal sand on the floor of the mangrove forests.

The tsunami damaged many human lives and much property in coastal areas as well as natural ecosystems (MNRE 2005; UNEP 2005; Stoddart 2007), mainly in fishing villages and beach resorts facing the Andaman Sea (Matsumoto et al. 2006). These areas were characterized by shallow sub-tidal beaches and/or closed-off section of bays and low, flat land. Around our survey plots, the tsunami waves reached approximately 6 m in maximum height above ground (information from RCRRS staffs) leading to damage of mangrove ecosystems. Damage to mangrove forests and macrobenthic communities was limited and habitat specific. Although benthic organisms decreased temporarily just after the tsunami (Plots A and B), the community recovered quickly.In highly disturbed areas (Plot C) where thick sand accumulated on the bottom of the mangrove forest, recovery appears to be difficult due to altered sediment composition and depth.

Tsunami-related damage to the mangrove ecosystem was not only direct physical damage, but also subsequent decline/death/change due to salt stress, drying stress, etc., as has been discussed in some reports (MNRE 2005; UNEP 2005; Matsumoto *et al* 2006; Stoddart 2007). It is important to continue monitoring to evaluate the recovery process of mangrove forests and macrobenthic communities from the catastrophic damage.

Acknowledgement
We are grateful to the staff of Ranong coastal resources research station, Kasetsart University, for supporting the field survey. This project was partly supported by Grants-in Aids for Scientific Research from Japan Society for the Promotion of Science and Grant Program of the Sumitomo Foundation. We are also grateful to Dr. Clive Wilkinson for his valuable suggestions and editorial help to improve the manuscript.

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Rapid recovery from bleaching events - Fiji Coral Reef Monitoring Network Assessment of hard coral cover from 1999-2007

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Abstract. Long-term biological monitoring of coral reef sites across the Fiji Islands was carried out over a nine year period which included mass coral bleaching events. Sites were fringing reef slopes close to shore, or patch reefs facing deeper water, and surveys were carried out between 5 and 20m depth. Data was contributed by many organisations, including scientists, tourism operators, non-governmental organisations and community members. All surveys utilised point intercept transects from which the percentage of hard coral cover was applied for regional comparisons over-time. Benthic data was generally recorded as coral life-form categories. High sea surface temperatures (SST) in 2000 and 2002 led to large-scale coral bleaching events with hard coral mortality of 40 – 80%. In both cases, temperatures remained above the long-term summer average (28.3°C) for over 3 months. Though variable, substantial recovery to pre-bleaching levels was seen within 5 years in many areas. Fiji's reefs show great diversity, and are spread over a large geographical area. Though mass bleaching events were expansive, they did not affect the entire country's reef systems at one time. Some areas and habitats appear to have elements conducive to minimising the effects of bleaching. This allowed for the repopulating of affected areas more swiftly than may occur in reefs that are more isolated. A high level of resiliency of the Fiji reef system as a whole is indicated and is a cause for optimism.

Key words: coral reef monitoring, coral bleaching, resilience, Fijian coral reefs

Introduction

Coral reefs have been characterized as degrading worldwide (Indo Pacific (Wilkinson 2008); SE Asia (Burke et al. 2002); Caribbean (Gardner et al. 2003)). While many areas with high human population density are greatly affected by human-related impacts, in more remote areas climate change is a more immediate issue, and coral bleaching events are amongst the main factors contributing to wide scale degradation. Coral bleaching events are the main factor contributing to the wide scale degradation, having impacted coral reefs globally. However, some coral reef regions have experienced rapid recovery after major bleaching events, and such is the case in Fiji (Lovell & Sykes 2004, Sykes 2007).

Relative resilience to the widespread mortality of coral reefs during a coral bleaching event is variable. In Fiji, the rapid recolonization of affected reefs may be due largely to the size and physical complexity of the archipelago. The Fijian archipelago spans 18,500km^2 and comprises high and low islands with all reef types including one of the world's longest barrier reef (South and Skelton 2000, Vuki et al. 2000). It is a mid-ocean archipelago surrounded by deep water and washed by oceanic currents.

This paper represents a summary of annual reports submitted by the Fiji Coral Reef Monitoring Network (FCRMN) as part of its contribution to the Global Coral Reef Monitoring Network (GCRMN) and Reef Check. Data was collated from a number of organizations which include scientists, tourism operators, non-governmental organisations and community members. The programme is coordinated by the authors and the Institute of Marine Resources. It is currently supported by the University of the South Pacific and the Coral Reef Initiative in the South Pacific (CRISP).

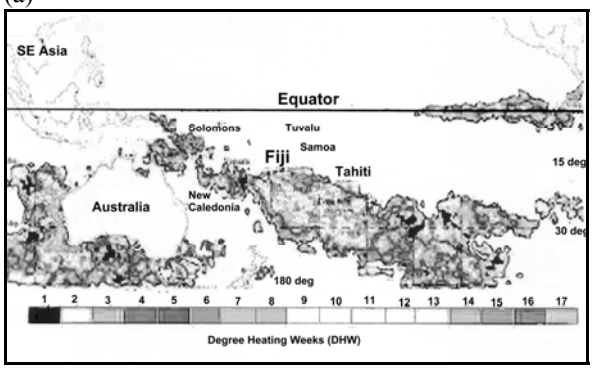
This paper summarizes the results of nine years of biological monitoring, using the Reef Check and FCRMN Point Intercept Transect (PIT) methodologies.

Background

Fiji SSTs range annually between 24°C and 28°C, with a bleaching threshold at 29.2°C (National Oceanic and Atmospheric Agency (NOAA) NESDIS satellite information).

High SSTs in 2000 and 2002 led to large scale coral bleaching events with hard coral mortality of 40-80% in depths <5m. In both cases, the temperature remained above the long-term mean and the bleaching threshold for 3 months with a band of warm water extending from the Solomon Islands to the southeast, either partially or completely covering the archipelago (Cummings et al. 2001, 2002; Lovell and Sykes 2004).

(a)



(b)

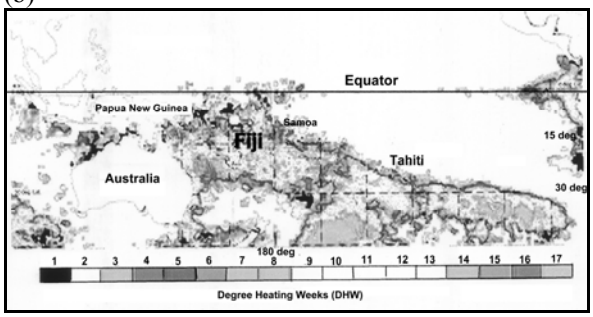


Figure 1. Chart of Degree Heat Weeks (DHW) accumulated in April, 2000 (a) and in 2002 (b) in the South Pacific. Light shading indicates 8-10 DHW's, darker shading inside of the light shading indicates 10-14 DHW's. See NOAA website: http://psbsgil.nesdis.noaa.gov:8080/PSB/EPS/SST/dhw_retro.html. National Oceanic and Atmospheric Agency (NOAA) NESDIS satellite information.

Methodology

Benthic data was assessed using point intercept transects, with the benthic attributes recorded as coral life-form categories or species. In all cases, the information was gathered over a period of years, although the most detailed and longest term data came from two areas, Suva and the central Vatu-i-Ra Passage. In-situ temperature loggers were placed in sites where biological surveys were made.

Results

In-situ temperature loggers showed a North-South sea water temperature variation of 1.5^{o}C across the archipelago (Figure 4).

For depths of 10m and deeper (<20m), benthic assessment showed a consistent trend of declining hard coral cover after the 2000 and 2002 mass coral

a)

b)

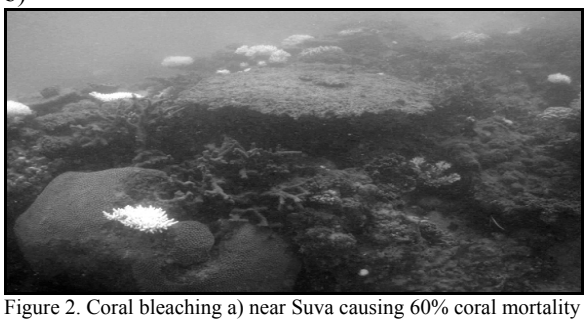


Figure 2. Coral bleaching a) near Suva causing 60% coral mortality in 2000 and b) at a reef near Vatu-i-Ra Passage.

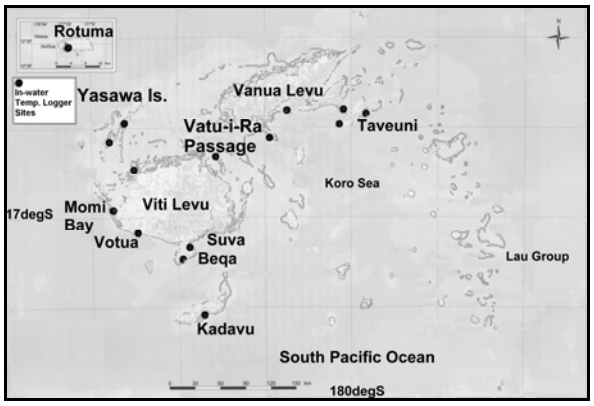


Figure 3. Chart of the Fiji archipelago showing the survey and temperature recording sites. Adapted from Spalding, Ravilious and Green (2001).

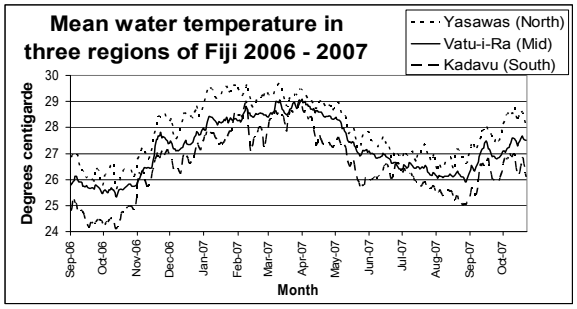
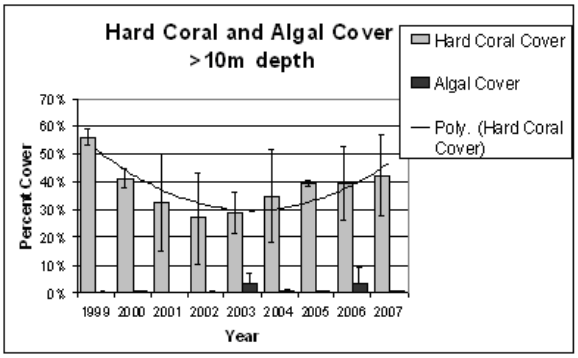


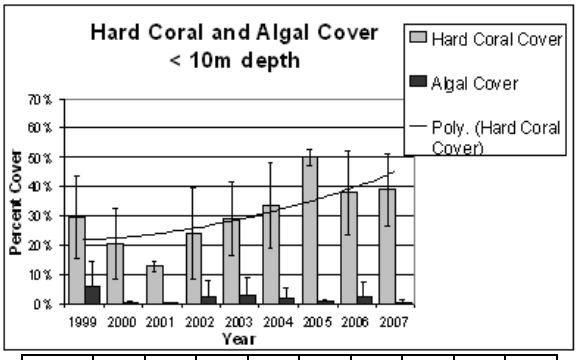
Figure 4. Graph of mean sea water temperatures at depths of 5 - 10m at three sites across the Fijian Archipelago (Vemco Mini-loggers set at 2 hour intervals).

bleaching events. An increase in coral cover was evident by 2003, and by 2007 was approaching the 1999 level (Figure 5). For depths less than 10m, by 2005, the degree of coral cover exceeded the coral coverage assessed in 1999 (Figure 6). As was characteristic of all reefs, algal cover remained low except in areas where anthropogenic effects, such as nutrient pollution and overfishing, have occurred (Mosley and Aalsberberg 2003).



Year	99	00	01	02	03	04	05	06	07
N	2	2	3	6	5	10	2	16	15

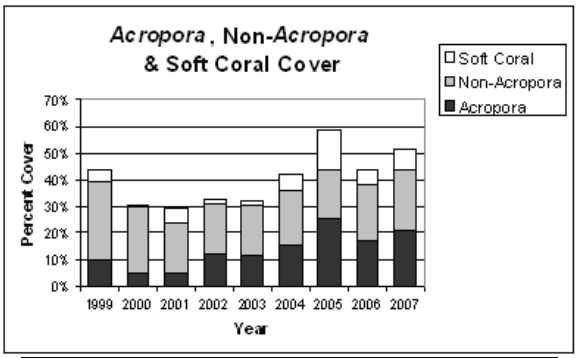
Figure 5. Mean hard coral and algal cover on reefs deeper than 10 m, across the Fiji Islands 1999 – 2007. Error bars show standard deviation with polynomial trend lines, Order 2.



Year	99	00	01	02	03	04	05	06	07
n	3	3	2	12	15	15	5	17	17

Figure 6. Mean hard coral and algal cover on reefs shallower than 10 m across the Fiji Islands 1999 – 2007. Error bars show standard deviation with polynomial trend lines, Order 2.

Figure 7 shows the breakdown of coral types into *Acropora* or non-*Acropora* and soft coral over the 9 years of assessment. The bleaching phenomenon in 2000, 2002, and subsequent crown of thorns seastar predation and cyclone damage had greater impacts on *Acropora* corals than on non-Acropora and soft corals. Differences in trends between Figures 5-6 and Figure 7 are due to the combination of data from all depths in Figure 6.



Year	99	00	01	02	03	04	05	06	07
n	5	4	5	8	10	15	3	26	25

Figure 7. Mean hard coral cover across the Fiji Islands 1999 – 2007, showing separation into coral types (Acropora, non-Acropora and soft coral) combining all depths.

Figure 8. summarises the effects of coral bleaching at one site from 1999 – 2007. There was 60% live coral coverage in the Vatu-i-Ra passage in 1999. In 2000, 42% had died or were bleached. In subsequent years, partial bleaching occurred but the general level of coral coverage increased.

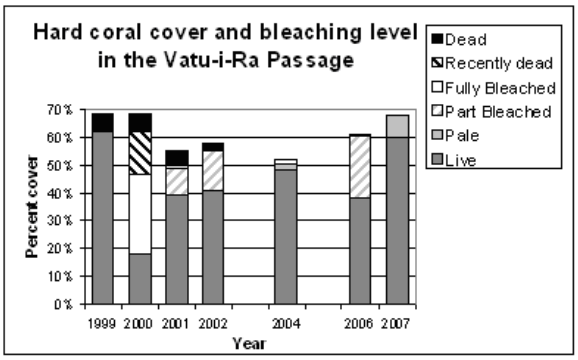


Figure 8. Hard coral cover and extent of bleaching at time of survey in the Vatu-i-Ra Passage (Mount Mutiny) 1999 – 2007. No surveys were carried out in 2003 and 2005.

In Figures 9 and 10, coral types using lifeform categories (English et al. 1997) are shown for the years 1999 – 2007, for a single site and as the Fiji-wide mean. In both cases, *Acropora* coral forms were more severely affected by bleaching than non-*Acropora*, but had recovered to pre-bleaching levels by 2005. Post bleaching reefs showed a higher diversity of coral lifeforms than pre-bleaching.

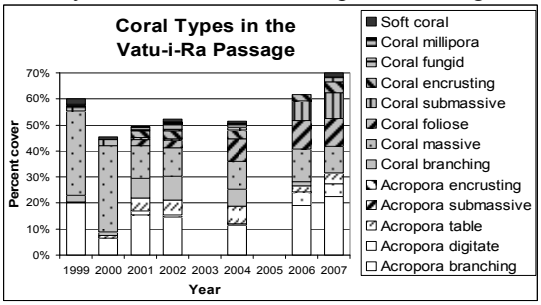
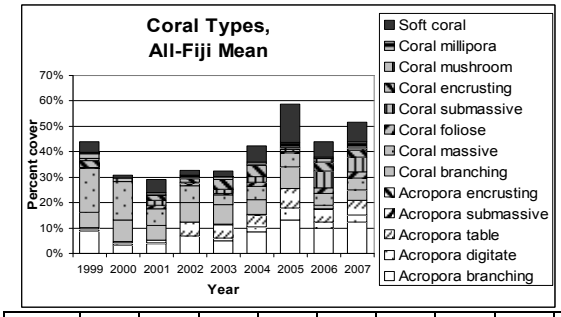


Figure 9. Mean main coral types in life-form categories at the Vatu-i-Ra Passage site 1999 – 2007. No surveys were carried out in 2003 and 2005.



Year	99	00	01	02	03	04	05	06	07
n	5	4	5	8	10	15	3	26	25

Figure 10. Mean main coral types in life-form categories for all sites in Fiji during the period of 1999 – 2007.

Discussion

In 2000 and 2002, Fiji's coral reefs experienced extensive bleaching mortality, with a total loss of between 40 and 80% of hard coral across the archipelago. The worst affected corals were largely from the *Acropora* family, which in some areas were completely obliterated.

For a two to three year period after the 2000 bleaching, many reefs were dominated by non-*Acropora* corals, predominantly *Pocillopora*, *Porites* and *Montipora* species, but *Acropora* corals reappeared after a very short hiatus, and by 2004 equalled or exceeded pre-bleaching levels.

By 2007, coral cover was substantial on most reefs, recovering to a national average of 45%, with up to 80% live hard coral cover in some regions. In many areas recovered with hard coral cover and lifeform diversity in 2007 was higher than pre-2000 bleaching levels.

In many cases reefs which recovered quickly were down-current of unaffected areas, and so were probably connected to sources of new coral spawn. Recovery in some areas appeared to be accelerated by a cyclonic event which cleared many reefs of dead algae-coved rubble, creating new coral settlement habitat. Where recovery was slow, probable causes include physical remoteness or algae domination preventing coral settlement.

While the coral bleaching events were extensive they did not affect the entire country's reef systems at one time, and though variable, substantial recovery to pre-bleaching levels was seen within 5 years, in many areas indicating a high level of resiliency to bleaching events. Several factors are thought to be integral to this resiliency.

Fiji's reefs show great physical and species diversity and are spread over a large geographical area. Reservoirs of unaffected coral were always to be found somewhere in the archipelago.

While Fiji's sea water temperatures have reached or exceeded the bleaching threshold in most summers of the last 10 years, there is a sea water temperature range of 1.5^{o}C over the archipelago. This range provides areas where corals exist in lower temperature water, and appear to have escaped the mass bleachings.

In addition Fiji is remote from highly populated landmasses and large scale industrial pollution sources. There are threats to near-shore reefs from agricultural pollution, coastal development, and over-fishing, but there are extensive reefs areas unaffected by these.

As a result of combinations of these factors, corals have managed to survive the mass bleaching mortality events of 2000 and 2002 in habitats which appear to be conducive to minimizing the effects of bleaching. This allowed for the repopulating of affected areas more swiftly than may occur in more isolated reefs.

Conclusions

After 9 years of monitoring including the 2000 & 2002 bleaching events, Fiji's reef system experienced rapid coral re-growth in many areas.

Over the country, many reefs returned to pre-bleaching coral cover levels in approximately 5 years. Fiji corals can survive catastrophic events as long as they do not occur too often. A high degree of resiliency of the Fiji reef system as a whole is indicated, and is a cause for optimism.

There may be a case for special attention to preservation of remote yet connected reef systems such as Fiji to provide reservoirs of resilient corals in the face of the continuing global downturn in coral reef health.

Acknowledgements

Appreciation for the Global Coral Reef Monitoring Network (GCRMN) and Reef Check for initiating a co-operative project to train a network of survey teams who could report on coral reef health across the country. Many thanks to the Fiji Coral Reef Monitoring Network participants whose data is summarized in this report. Coordination for the project was provided by the Institute of Marine Resources at the University of the South Pacific and Resort Support, Fiji. The Coral Reef Initiative in the South Pacific (CRISP) provided funding for this project.

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Socioeconomic Monitoring (SocMon) as a Tool in the Management of Marine Protected Areas: Participatory Process and Initial Survey Results in Binduyan Fish Sanctuary, Palawan, Philippines

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Abstract. Tools in the assessment of socioeconomic and governance factors for managing marine protected areas (MPAs) are continuously being developed. A standardized set of guidelines to conduct socioeconomic monitoring for collecting and analyzing socioeconomic was developed and dubbed as the Socioeconomic Monitoring Guidelines for Coastal Managers in Southeast Asia (SocMon SEA). SocMon SEA was employed to assess the dependence on marine resources, perceptions of resource conditions, perceived threats to marine resources and use levels at Binduyan Fish Sanctuary, a locally-managed MPA in Puerto Princesa City, Palawan Province, Philippines. Results of the survey indicate that almost half of its population is below 20 years old; nearly three quarters are migrants; a quarter reached high school level; and barely 12% belong to a stakeholder organization. These stakeholders perceived their natural resources to be in good condition. They also articulated their perceived threats related to cutting of mangroves, destructive fishing activities and pollution for beaches. These informative results indicate that SocMon SEA as a methodological tool was useful in generating relevant information in evaluating the current socioeconomic conditions in an MPA. Such demonstrates its potential for large-scale use in the Philippines and Southeast Asia due to its ease in field application and data analysis.

Key words: marine protected areas (MPAs), Socioeconomic Monitoring Guidelines for Coastal Managers in Southeast Asia (SocMon SEA), Binduyan Fish Sanctuary, Puerto Princesa City, Palawan Province, Philippines.

Introduction

Methodological tools in the assessment of biophysical parameters in marine protected areas (MPAs) have been fairly standardized. The same level of consensus is not yet reached in assessing the human dimension's socioeconomic and governance variables. In this context, 'Socioeconomic Monitoring Guidelines for Coastal Managers in Southeast Asia (SocMon SEA)' was developed. SocMon SEA is a standardized set of guidelines on how to conduct socioeconomic monitoring for coastal managers. SocMon SEA is intended to be a simple methodology for collecting and analyzing basic socioeconomic data useful for coastal management at the site level (Bunce and Pomeroy 2000). SocMon is a companion to the GCRMN Socioeconomic Manual for Coral Reef Management (Bunce et al 2003). Both the SocMon SEA and the GCRMN manual should be used together.

Understanding the socioeconomic context of any MPA is essential for assessing, predicting and managing coastal resource use. Without or with limited socioeconomic information, the coastal managers will be constrained in planning and managing their coastal resources. SocMon SEA is now being used in many parts of South East Asia to enhance understanding of the social, cultural, economic and political characteristics. Moreover, it helps in assessing the conditions of individuals, households, groups, organizations, and communities who use coastal resources and are affected by coastal management.

SocMon SEA was specifically employed in this Philippines' case study to: (1) establish baseline household and community profiles for monitoring and evaluation; (2) determine the importance, value and significance of resources and their uses; (3) build stakeholder participation; and (4) train researchers on SocMon SEA's field survey methods and data analysis. This paper presents some of the results generated at the Binduyan Fish Sanctuary, a locally-managed MPA situated in Puerto Princesa City,

Palawan Province, Philippines. One of the constraints identified in the management of this MPA site is the limited relevant socio-economic baseline information. Hence, from the management context, the SocMon was used as both a participatory and research tool to generate some of the desired sets of information.

Material and Methods

Methodologically, SocMon SEA involves a three-stage process (Figure 1). Advance preparation involves several activities including identifying the coastal stakeholders, identifying the study area, assembling a SocMon team, identifying the variables to be examined and obtaining government and stakeholder approvals. Data collection is at the core of the SocMon process, ideally consisting of 60 socioeconomic variables. It involves both secondary data analysis and primary data gathering. Report writing follows after data analysis. The results are then packaged and communicated to the relevant stakeholders.

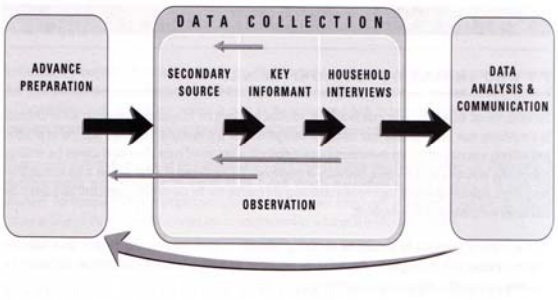

Figure 1. Major phases for conducting SocMon. (Source: Bunce and Pomeroy 2003)

Binduyan Fish Sanctuary, a locally-managed MPA situated in Puerto Princesa City, Palawan Province, Philippines, was selected as the study area (Figure 2 and Figure 3). Consisting of a corraline area, with associated mangroves and seagrass beds, it was declared as an MPA in 2002. Its reef area had about 48% live coral cover in 2004. In 2008, the live coral cover was assessed at 63%.

Binduyan is typical of most coastal fishing villages in the Philippines (Bernardo and Valientes 2001; Environmental Legal Assistance Center, Inc 2001; Office of the City Agriculturist. 2001). Founded in 1960, its land area covers some 8,968 ha. With about 1,200 people clustered in 193 households, there were an almost equal number of male and female residents. Its population is highly productive, given that 55% are below 20 years old. Barely 12% belong to stakeholder organizations. Migrant population now comprise nearly three quarters of the residents. Community members are heavily dependent on the fisheries. More recently, eco-tourism activities – such as island hopping and reef snorkeling – are being undertaken.

Figure 2. Location map of Puerto Princesa City, Palawan Province, Philippines.

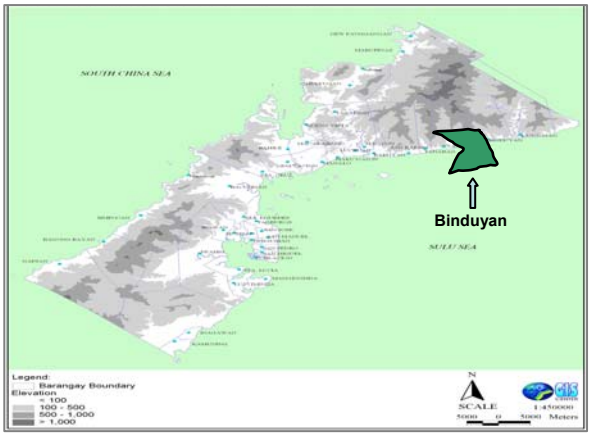

Figure 3. Location map of Binduyan Fish Sanctuary in Puerto Princesa City, Philippines.

The SocMon team assembled consisted of members from various institutional partners. Those who participated were representatives from a national government agency (Palawan Council for Sustainable Development Staff); a non-government organization (Conservation International-Philippines); a local academic institution (Palawan State University) and a local government unit (City Government of Puerto Princesa). The review of secondary literature, which was compiled from a variety of sources, was jointly undertaken by the SocMon team. Secondary literature consisted of biophysical, socioeconomic and governance information.

The SocMon team members collected primary data from two sources. The first set was obtained from the key informants. These were individuals who - because of their position, experience and/or knowledge - were able to provide insights into the larger population. The second source of information was from household/individual interviews. Important for understanding individuals'

perspectives, data were collected directly from the individual/household members. Only data from household/individual interviews are presented in this paper.

Results

Three key results are presented. These relate to the following socioeconomic variables: (1) perceptions of resource conditions; (2) perceptions of non-market and non-use values; and (3) material style of life of households.

Coastal residents generally perceived their resources to be in 'good' condition, with only very few individuals who perceived them in either 'bad' or 'very bad' condition (Table 1). They perceived their waterfalls to be in 'very good' condition with the highest mean rating at 4.21. They gave lowest ratings on seagrass, river/creeks, and upland forests with mean ratings hovering between 3.89 to 3.94.

Table 1. Perception of resource conditions. (n = 135)

Type of Resource	Perception Scores (%)*				
	1	2	3	4	5
I. Coastal					
1. Mangroves	2.3	2.3	10.5	59.4	25.6
2. Coral reefs	2.3	0.8	8.4	58.0	30.5
3. Seagrass		2.4	21.0	62.1	14.5
4. Beach	0.8	2.5	8.2	51.6	36.9
II. Terrestrial					
1. Spring	2.8	1.9	19.6	43.0	25.0
2. River/Creeks		3.4	25.6	46.2	24.8
3.Waterfalls	1.1	1.1	12.9	33.3	51.6
4.Ground Water	6.0	2.6	13.7	40.2	37.6
5. Upland forests		2.3	26.7	45.8	25.2

*Very bad – 1; Bad – 2; Neither good nor bad – 3; Good – 4; Very Good – 5

Resources are conveniently divided into coastal and terrestrial. Hence, their perception ratings may be aggregated by resource category. Coastal resource conditions had a mean rating of 4.09. Meanwhile, terrestrial resource conditions had a mean rating of 4.07. Standard deviations are relatively small at 0.46 and 0.52, respectively. Hence, both resource categories fall within the 'good' rating.

Factor analysis was undertaken to aggregate the responses of respondents. Rotated component matrix of the factor analysis reduced the perceptions on conditions of nine resources into four factors (Table 2). Those with asterisks are the ones that have high and logical factor loadings on each component. Respondents' perceptions on the conditions are almost similar for: (1) spring and river/creeks; (2) coral reefs, seagrass, beach; (3) waterfalls, ground water; and (4) mangroves and upland forests. The first three clusters appear logical, as similar resources are clustered together. The fourth cluster is interesting, as the respondents seem to view mangroves as not part of the coastal ecosystems. Rather, they treated the mangroves as more of terrestrial forests, similar to those found in the uplands. Some 73.3% of the variance in perceptions is explained by the four extracted factors.

Table 2. Rotated component matrix of the factor analysis of perception of resource conditions (n = 135).

Resource	Component			
	1	2	3	4
Mangroves	.537	.155	-.334	.438*
Coral reefs	.352	.601*	.024	.130
Upland forests	.013	.058	-.093	.855*
Seagrass	-.078	.867*	.114	.146
Beach	.394	.728*	-.251	-.241
Spring	.868*	.202	.087	-.020
River/Creeks	.830*	.053	.327	.020
Waterfalls	.220	-.020	.862*	-.208
Ground Water	.063	.510	.523*	.412

Residents valued their coastal resources far beyond their direct use values (Figure 3). Majority of the respondents perceived the coastal resources as more than products to be bought or sold in the market. Hence, the resources are significant to them on any of the following: (1) resources have value for its potential future direct and direct use (option value); (2) resources have value if it will be available for future generations to enjoy or utilize (bequest values) or, (3) value of knowing that the resource subsist in a certain condition whether it is utilized or not (existence value). Overall more than of two thirds of the respondents perceived that the coastal resources have value irrespective of whether it is used directly or indirectly.

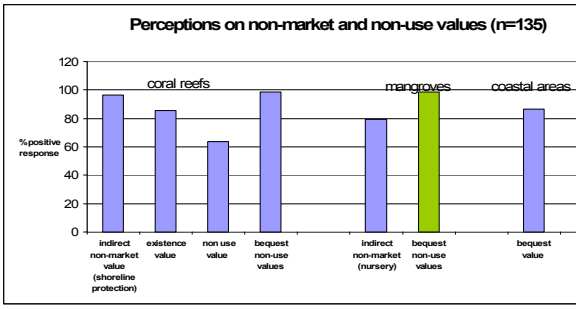

Figure 3. Perception of non-market and non-use values resource conditions. (n = 135)

Wealth is one of the most difficult indicators to measure in developing countries. Hence, 'proxy' variables are often used to determine the household level of wealth or income. 'Material style of life' is an indicator of the relative social status of a community household to indicate the level of wealth its members.

It involves assessing both the household construction materials and the household furnishings and electronics contained therein.

Houses of Binduyan residents are typical of rural coastal households (Table 3). Roof are either made up of any of these materials: (1) thatch/nipa (made of palm leaves) and tin/galvanized iron (GI) sheet; (2) outside structural walls consist largely of thatch/bamboo, brick/concrete and wood plywood; (3) wooden windows; and (4) cement or thatch/bamboo floors.

Table 3. Material style of life of households. (n = 135)

Parameter for Material Style of Life	Value	%
Type of Roof		
Tile	1	0
Tin/GI sheet	2	45.6
Wood/Plywood	3	0.9
Thatch/nipa	4	52.6
Thatch/bamboo	5	0.9
Type of outside structural walls		
Tiled	1	0
Brick/concrete	2	18.9
Wood/Plywood	3	18.9
Thatch/nipa	4	12.2
Thatch/bamboo	5	50.0
Windows		
Glass	1	7.9
Steelbars/grills	2	1.8
Wooden	3	67.5
Open	4	15.8
None	5	7.0
Floors		
Tile	1	2.6
Wooden	2	3.5
Cement	3	42.6
Thatch/bamboo	4	42.6
Dirt	5	8.7

Each of the responses was converted into numerical rating: in the case of the type of roof, for example, tin/GI sheet has a value of 4, while thatch/bamboo has a value of 1. As an instance for windows, a glass window has a value of 1, while a wooden window has a value of 3. Out of a lowest possible aggregate score of 5 (very high material style of life) and highest aggregate score of 20 (very low material style of life), the computed scores ranged from 8 to 18. This range was transformed into three categories: low, middle, and high. The aggregated ratings for 'material style of life' are as follows: low 30.4%; middle 36.3%; and high 33.3%.

Discussion

Community residents perceived that that the conditions of their terrestrial and natural resources are still generally in good condition. These community perceptions are largely consistent with biophysical monitoring of both coastal and terrestrial resources. They admitted, however, that there are continuing threats associated with development activities. Major threats to mangroves include cutting for household uses and clearing for human settlements. Threats to corals are mainly associated with illegal fishing activities, particularly the use of cyanide, compressor and dynamite. Threats to seagrass beds largely relate to fishing using dragnets and gleaning. The key problem with beaches is garbage dumping or solid waste disposal; other threats are development-related, such as pebble gathering/taking, sand quarrying and beach resort development. Perceived threats in order of decreasing importance were: cutting of mangroves; destructive fishing activities and pollution for beaches. To maintain the relatively good conditions of the natural resources, the threats identified must be properly mitigated with appropriate management actions/interventions.

The community members are aware of the benefits of the coastal resources beyond direct use values. They generally agree about the indirect value of the resources that are not traded in the market. They also put high value on future benefits or option value for future generation. The MPA's values for eco-tourism and aquaculture are now being given importance. For eco-tourism, related activities include diving, snorkeling and swimming. In the case of aquaculture, environment-friendly seaweeds farming are being promoted in the vicinities to augment the income of the community members.

The use of proxy variables – such as the material style of life – was an innovative part of the SocMon SEA methodology. In rural setting, wealth is one of the most difficult socioeconomic variables to measure. Through the SocMon process, we were able to approximate the relative level of wealth of the Binduyan households. Generally speaking, the economic strata of the household units can be arbitrarily classified into three groups: one third is wealthy; one third is middle class; and one third is poor. The local authorities are now aware of the economically-disadvantaged group to target for their future socio-economic interventions.

SocMon SEA was found useful in generating relevant information to evaluate the current socioeconomic conditions in MPAs. These include information on demographics, local perceptions, attitudes and values about natural resources. The results of the study are now being used in local development planning process specifically at the city and community level. Moreover, the SocMon SEA has recently been used in generating some socio-economic information of two coastal villages within Puerto Princesa City.

SocMon SEA has potential for between and across site comparisons, given the SocMon surveys undertaken in the provinces of Palawan and Batangas. It has also potential for large-scale use in the Philippines and Southeast Asia by virtue of its ease in field application and data analysis.

Acknowledgement

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Reefs at risk in Central Sulawesi, Indonesia - status and outlook

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Abstract. Central Sulawesi Province, Indonesia in the heart of the Coral Triangle with over 4,500km of coastline including the Banggai Archipelago, Togean Islands Park and several smaller MPAs. Since the Reefs at Risk study in 2000/2001 predicted high threat levels for most reefs in the Province, several survey and monitoring programmes have been supported by international, national and local sources. This paper summarises coral reef condition and socio-economic data over the period 2001-2007 in 7 of the 10 District/City areas, key conclusions and local initiatives. Based on the Global Coral Reef Monitoring Network (GCRMN) scale, the average condition is Poor, with reefs in Very Poor, Poor, Average and Good condition in all 7 areas but extremely limited areas in Very Good condition. Major impacts include coral mining, sedimentation, destructive fishing (including invertebrate collection.), increasingly severe overfishing, take of protected species and predation by *Acanthaster plancii*, with low awareness regarding many illegal and/or destructive practices. Initiatives include coastal/reef ecology, survey and conservation for undergraduates, community MPAs, COTs clean-up and habitat restoration. However the extent and scope of management efforts still needs to be greatly increased to reverse the destructive trends and ensure "Reefs for the Future" here.

Key words: Central Sulawesi, Reef status, Reef monitoring, Reef management

Introduction and Methods

Central Sulawesi Province, Indonesia in the heart of the Coral Triangle has over 4,500km of coastline and over 700 islands including the Banggai and Togean Archipelagos, with almost continuous fringing reefs, extensive barrier reefs, patch reefs and several atolls.

The Togean Islands National Park was declared in 2004 and there are several smaller national and local MPAs. Since the Reefs at Risk study in 2000/2001 (Burke et al. 2001) predicted high threat levels for most of the reefs in the Province, a number of survey and monitoring programmes have been undertaken using GCRMN methods: Reef Check (Hodgson et al., 2004); manta tow, Line Intercept and Point Intercept transects (LIT/PIT) (English et al., 1997) in 7 of the 9 Districts and in Palu City, with support from international, national and local sources (Fig. 1.).

Many of these surveys also collected socio-economic data, mainly using the KAP (Knowledge, Attitude and Perception) method (CRITC 2001) or livelihoods survey tools (STREAM 2002). Published survey data and official reports (listed under References) are supplemented with as yet unpublished data and information collected by the authors.

The data available was analysed for two geographical areas: the so-called "West Coast" facing the Makassar Straits and Sulawesi Sea, including the Donggala, Tolitoli and Buol Districts and the City of Palu, and the "East Coast" around the Gulf of Tomini and Gulf of Tolo, including the Districts of Parigi Moutong, Poso, Tojo Una-Una, Banggai Kepulauan, , Banggai and Morowali.

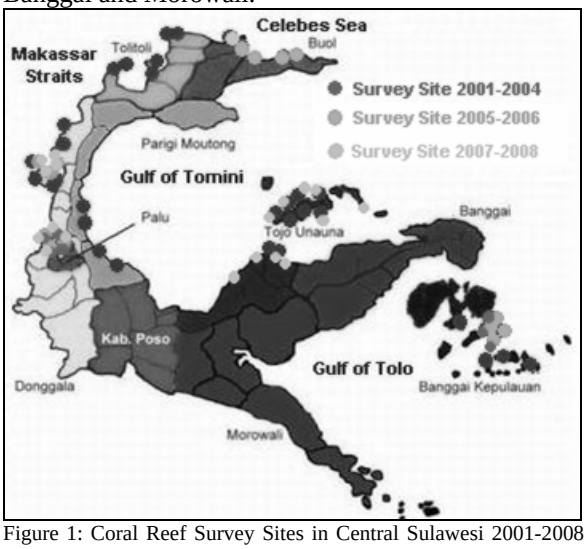

Figure 1: Coral Reef Survey Sites in Central Sulawesi 2001-2008 for which data was available for this study

West Coast: Makassar Straits and Sulawesi Sea

Tolitoli and Buol Districts form part of the Sulu-Sulawesi Marine Ecoregion (SSME) where Indonesia, Malaysia and Philippines have signed an agreement as basis for integrated sustainable management,. However so far there has been no involvement of regional (District and Provincial) Governments or stakeholders.

Where time series are available there has been little change in overall condition since 2004, though there is a slight downward trend. The average condition is Poor (11-30% hard coral cover), with highest coral cover generally observed near the reef crest except at Pasoso MPA where corals thrive to depths below Reef Check standards or even safe diving limits.

The most recent Manta Tow data indicating the range of coral reef condition by District and Reef Check/PIT data indicating hard coral cover at sites in this area are shown in Fig. 2.

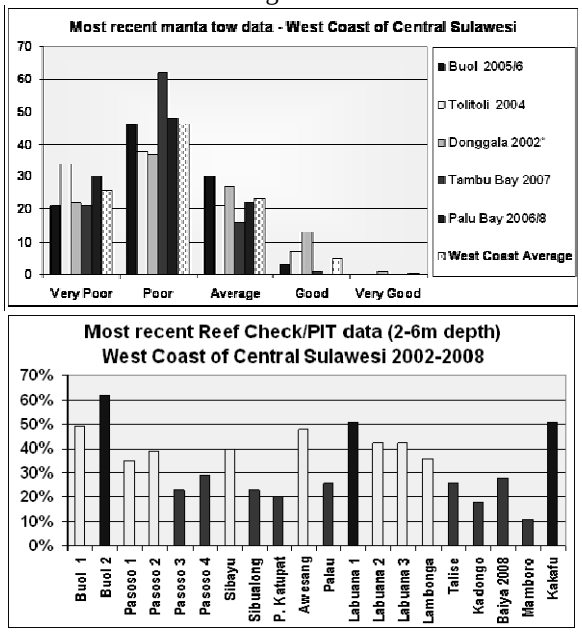

Figure 2: Most recent West Coast coral condition data, Manta Tow (above) and Reef Check/PIT (below)

Large fish are increasingly rare though some large demersal fish were seen in Buol. The Reef Check data from survey sites in the Makassar Straits and Palu Bay generally indicate very low populations of most commercial fish and invertebrate species.

KAP data from 2007 confirms this, with almost all fishers perceiving a fall in catch per unit effort (CPUE). In Tambu Bay, previously a source of fish for Palu City (Anonymous 2002) most fish sold is now from Kalimantan or the Gulf of Tomini (Anonymous 2007a)

Almost all marine species protected under the Law PP No. 7 1999 are found in the area and apart from the Coelacanth (recently discovered in Buol) all are exploited, most of them heavily. As an example, in Tambu Bay fishermen say the triton *Charonia tritonis* and napoleon wrasse *Cheilinus undulatus* are now fished out and turtles are increasingly rare; and all six species of Tridacnidae are collected in huge numbers and sold at low prices (cheaper than fish) in Tolitoli.

COTS attacks: the corallivorous Crown of Thorns starfish *Acanthaster plancii* is a major problem in all areas surveyed from 2004 onwards, a trend which was predicted from 2002 data (Anonymous 2002)

Coral mining: although illegal, massive corals are sold openly for around $10/m3. According to community members law enforcement personnel are often involved actively or in collusion

East Coast: Gulf of Tomini and Gulf of Tolo

Tomini Gulf data were available for Parigi Moutong and Tojo Una-Una, and Tolo Gulf data was from Banggai Kepulauan, whereas no data were available from Poso, Banggai, and Morowali Districts, meaning coral reef data for the Sulawesi mainland were limited with none for the Gulf of Tolo.

Banggai Kepulauan District comprises the Banggai Islands, and most coral reef data were from habitat and population surveys of the endemic Banggai cardinalfish, *Pterapogon. Kauderni*, there were no Reef Check indicator species data. However recorded observations and KAP study results indicate that overfishing is generally less severe than in the Gulf of Tomini or the West Coast. The abundance of fisheries produce has prompted the establishment of an industrial-scale fish processing plant at Biak in the Gulf of Tolo.

The average condition is (just) Average, with more Good reefs seen than on the West Coast (Fig. 3).

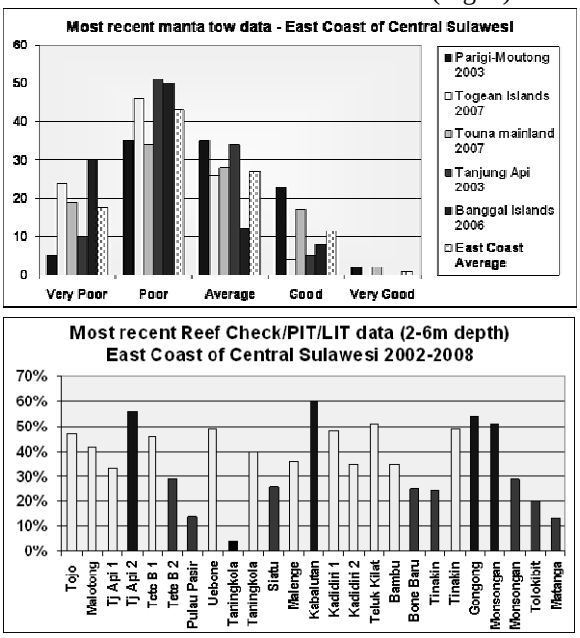

Figure 3: Most recent East Coast coral condition data, Manta Tow (above) and Reef Check/PIT (below)

Near to the shore, including the Togean Islands National Park, there is much severe damage due to

broadcast use of poisons (including cyanide), coral mining, gleaning etc, whereas bomb damage is generally worst at remote sites and mechanical damage when gleaning or harvesting invertebrates with crowbars especially abalone (*Haliotis* sp.) is often even more severe than the effects of better known forms of illegal and destructive fishing.

COTS: *A. plancii* is causing major damage at sites in Banggai Island (2004-2007) and was observed in high number at some sites in Tojo Unauna in (2008), other locations may be affected.

Ornamental fish trade: high in the Banggai Islands, increasing in Tojo Unauna and Parigi Moutong, as is the live reef fish trade which is prevalent in the area, with frequent use of poisons and mechanical damage.

Sedimentation is severe near larger rivers, high seasonal discharges seem to have killed some reefs near to estuaries, the remaining visible tips attesting to the recent nature of this phenomena. The majority of mangroves have been degraded or lost, according to a recent study comparing available historical data.

In most areas domestic garbage is a common sight, and in some areas severe eutrophication has been observed, especially near intensive paddy fields. However in the islands water quality is generally excellent, there is high marine biodiversity and despite extensive damage this area has several dive destinations with potential to expand, including reef conservation activities, "macro" photography and other specialty interests.

The process of establishing MPAs in Parigi-Moutong, Tojo Unauna and Banggai Kepulauan Districts has been facilitated by the communication of survey data to local stakeholders. Several de-facto reserves have evolved around marine tourism sites, often not without conflict.

General Trends and Threats

Some of the trends and threats observed across the Province include:

Coral condition monitoring: slight downward trend in coral condition; increased indirect impacts

Attitudes: KAP studies from 2002 and 2007 show growing community-level awareness regarding destructive fishing but little change as regards most other threats

Destructive fishing: bomb fishing: substantially reduced in some areas; poison fishing: reduced in some areas but increased in others, linked to the spread of the live and ornamental fish trades; other less-well known forms, especially related to invertebrate collection (abalone, clams etc), are often as damaging or even more so

Coastal abrasion: a growing problem in all areas, linked to the widespread coral mining & mechanical damage

Take of protected species: rife wherever economically worthwhile, sometimes due to ignorance but more often knowingly;

Overfishing: seems to be increasing

IUU fishing: many incursions by foreign vessels, mis/non-reporting of catch/cargo, etc

COTS outbreaks are occurring in all areas surveyed since 2004, causing substantial damage.

Recent initiatives and IYOR events

Initiatives since the presentation at the 10th ICRS in Okinawa in 2004 (Moore *et al*. 2004) have included:

Further dive/survey capacity building/training supported by the Sea Partnership Programme and Tojo Unauna Tourism Service.

The introduction of coastal ecology and conservation-related subjects into the curriculum of all local fisheries and marine undergraduate courses including field activities such as survey/monitoring and coral restoration trials

The establishment of several community MPAs (some proving effective) and the Togean Islands National Park (management to date ineffective with poor stakeholder relations)

Dive for Earth Day COTS control & reef monitoring (Reef Check, AWARE Fish Count, Coral Watch) in Palu Bay by YPH and STPL-Palu, supported in 2007 by Yayasan Reef Check Indonesia, in 2008 an IYOR event.

The establishment of the Tojo Una-Una Coral Triangle Centre (CTC) in February 2008 as a local response to the Coral Triangle Initiative (Fig 4.)

Figure 4: Declaration of the Tojo Unauna CTC in the Togean Islands by the District Head Drs Damsik Ladjalani.

Some IYOR activities have already taken place, however further events planned to celebrate the second IYOR in Central Sulawesi include:

Activities in connection with the Togean Festival and the Tojo Unauna CTC

"Fish homes" reef restoration in Palu Bay (Provincial Fisheries Service), Palu City and Donggala District (underway)

Biorock coral reef restoration training and application in Palu Bay (Provincial Fisheries Service) and Tojo Una-Una District (Sea Partnership Consortium and Tojo Unauna CTC)

Since 2004, a significant development is the promulgation of several national laws relating to coastal ecosystems, especially UU No27 of 2007 regarding coastal management, under which all activities which damage or destroy coral reefs are forbidden and most main threats to coral reefs are explicitly listed with heavy fines and prison sentences, including coral mining, at present possibly the number one direct threat to reefs in Central Sulawesi. However as far as the authors are aware no cases have yet been brought to court let alone resulted in conviction. At the local level, Provincial and District regulations for coastal management (PERDA Pesisir) and other instruments are in being drafted or even already promulgated, but similarly have yet to be (effectively) implemented.

Outlook for the future

Overall the outlook for reefs in Central Sulawesi is both better and worse than in 2004.

A positive point is the increase in awareness among stakeholders at community and government level including decision-making groups. However this is not always reflected in actions.

The improvements in the legal framework have yet to have a noticeable effect and law enforcement officials often seem to be among the least aware.

Government planning is beginning to be directed towards conservation and restoration and some local government authorities are beginning to implement programmes based on survey and monitoring data.

However the awareness of legislative bodies seems to lag behind that of the executive and in many cases have not ratified reef and other coastal system related budget items proposed by line agencies.

New threats are emerging. In particular, increasing threats from global climate change. Water temperatures range from 26-31°C, with 29-30°C being the most common, close to the upper tolerance limit for many coral species, though no significant bleaching has yet been recorded.

There is a long road ahead to ensure the protection of the reefs in good or average condition, stop or mitigate direct and indirect causes of degradation and restore damage. A major question is: can we increase the condition of our reefs and maximise their resilience to these new impacts, at a great enough scale and in time?

Acknowledgement

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Session number 18

Response of Caribbean Reefs to repeated Stress Events

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Abstract. In recent times, coastal ecosystems and specifically coral reefs have been experiencing varying degrees of stress from several sources. The incidence of natural phenomenons such as hurricanes and bleaching events has increased to the point where one or both of these events are guaranteed to occur annually. Coupled with effects of terreginous run-off and poorly planned coastal developments, the reefs of the region are constantly bombarded with less than ideal conditions. At present coral cover is still low at most sites assessed and has been for a long while.
In Jamaica Reef Check assessments indicating coverage ranging between 2% and 37% were recorded between 2001 and 2006. Results from bleaching assessments conducted during and after the 2005 event depicted up to 75% bleaching in some countries and varied levels of recovery. Data from long-term monitoring sites in Jamaica also produced results, which indicate that the reefs are to an extent able to survive and recover from the stress events. Given the increased frequency of stress events it is still unsure what level of recovery will be realized and the duration.

Key words: Caribbean, coral, stress, recovery

Introduction

The marine environment is the most important natural resource in Caribbean countries. Hurricanes, disease, climate change, population growth and overfishing have combined to exert enormous stress on reefs of the region. Terreginous run-off and poorly planned coastal developments result in the reefs being constantly bombarded with less than ideal conditions (UNEP 2008). Increased frequency and incidences of intense hurricanes, rising sea temperatures, coral bleaching, disease are now common dilemmas facing the islands (Fig. 1).

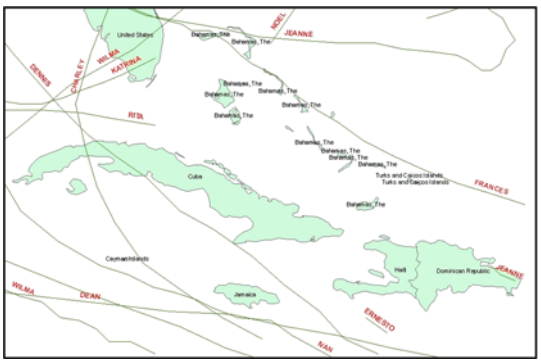

Figure 1 – Hurricane tracks 2004-2007

Current Status and Trends

The extent and severity of events varied across countries of the Northern Caribbean and Atlantic (NCA) node of the Global Coral Reef Monitoring Network (Fig. 2). As a result over the last quarter century some reefs associated with countries of the NCA node have experienced varied effects.

Figure 2 – Countries of the NCA node

Some corals were exposed to less stress and as a result were less susceptible to disease outbreaks. In the 2004-2005 bleaching outbreak between 50%-90% of coral colonies suffered partial to total bleaching. This resulted in a decrease of live coral cover in most islands and contributed to continued decline of the fishery of some islands (Souter and Wilkinson (Eds.) 2008).
In Bermuda the 2005 Atlantic hurricane season was the most active in recorded history with three tropical storms (Franklin, Harvey and Philippe) and two

hurricanes (Nate and Wilma). The increased frequency of hurricanes, repeated bleaching events and coral disease have had little impact on coral cover. Coral cover on the outer rim has been monitored since 1992 and has remained relatively stable over the 15 years (20.5% in 1992, 20.1% in 2007). Bermuda is the most northerly located country of the node. This may account for the fact that they do not experience prolonged periods of high water temperature which may cause bleaching.

The Bermudian fishery has experienced a shift towards pelagic species, particularly Wahoo (*Acanthocybium solandri*) and yellow-fin tuna (*Thunnus albacares*). Pelagic species now account for approximately 50% of the commercial finfish landings. This is in response to the ban imposed in 1990 on fish traps.

There is limited information on the current status of Bahamian reefs. Like other reefs of the region coral colonies are being pressured by proliferation of algae. Information from Rainbow Garden Reef suggests that bleaching, bio-erosion, and storm action reduced formerly discrete coral patches to rubble (Pante *et al*, 2007).

The Cayman Islands, specifically, Little Cayman recorded significant loss of coral cover between 1999 and 2004. There are however now signs of continued recruitment, decreased mortality and relatively stable coral cover. There has also been no increase recorded in the percent cover of fleshy macro-algae.

Episodes of coral bleaching were reported in the late 1980's, 1995, 1998, 2003, and 2005. The event was more pervasive in 2005 but did not result in mortality. From 1999 to present, there has been no overall change in the abundance or density of herbivorous fish species.

In Cuba, bleaching events of varying degrees were experienced in consecutive years during the period 2005 to 2007. Major stress events inclusive of frequent and intense hurricanes between 2001 and 2005, and microbial disease such as white plague, dark spot, white pox, yellow blotch and black band were common. Excessive algal proliferation due to scarcity of herbivores also affected several areas.

In the midst of these stress events a recovery of Diadema populations was observed along with resheathing on *Acropora palmata*. Reef Check surveys conducted in 2004 and 2005 indicated that hard coral mean percentage cover was 22.7% and 20.7% respectively. Atlantic Gulf Rapid Reef Assessment (AGRRA) surveys conducted in 2007 on the south and east of Golfo de Batabano provided live coral cover values of 9% to 50% for reef crests and 6% to 21% for reef zones.

The variation in reef status was again evident in the Dominican Republic. Reef conditions were dependent on the location and distance from the numerous river discharges. Reef Check assessments conducted between 2004 and 2007 showed that average coral cover ranged between 11.4% and 21.9%. Individual sites recorded percentages between 1.9% in 2005 and 34.4% in 2006.

The main problem on the reefs is the overfishing of several essential species such as *Strombus* sp., *Panulirus* sp., and fishes of the Serranidae, Lutjanidae, and Scaridae families. Additional threats include those of port development (construction and operations) at the Boca Chica reef which is the most studied reef site in the island. Increased sedimentation along the coast also threatens reef growth.

On the other half of the island of Hispaniola, Haiti, no comprehensive surveys have been conducted since 2003. At that time the reefs appeared to be in fairly good condition but, with no public sector involvement, the condition continues to degrade. Eutrophication, sedimentation, coral harvesting, pollution, and overfishing continue to be major issues affecting the coral reefs. At present the exportation of lobster and conch has been suspended by CITES. The unsustainable harvesting of wetland resources persists as mangroves are still being exploited for fuel wood, charcoal production and construction. Illegal coral harvesting continues and seagrass beds continue to be threatened by sedimentation and pollution.

The non-governmental organisation (NGO) Foundation pour la Protection de la Biodiversite Marine (FoProBIM) continues to monitor impacts and perform educational activities.

In 2005 the Turks and Caicos Islands like most of the region suffered a major bleaching event. As much as 50%-75% of coral colonies suffered partial to total bleaching. Reef Check assessments in 2004 and 2005 recorded averages of 10.6% and 5.9% respectively. In 2007 a more comprehensive assessment was conducted on 18 sites associated with the three major islands; Providenciales, West Caicos and South Caicos. Most sites assessed were dive sites with some level of legislative protection such as no anchoring and no fishing; depths between 12 and 60 feet were surveyed. On Providenciales live coral cover ranged between a low of 6.3% and a high of 38.8%. This is higher than the figures reported in 2004. Algal percentages ranging between zero and 46.3% were recorded. West Caicos and South Caicos recorded coral averages of 17% and 13% respectively.

Near-shore support systems such as seagrass beds and mangroves continue to thrive, however comprehensive monitoring of these systems is lacking. The country is currently experiencing a construction boom and all the associated pressures that arise from these activities.

Jamaican reefs continue to show variable cover by hard corals and other benthic substrates and have rebounded from the 5% hard coral cover recorded in the early 1990s to approximately 16%. Some sites have a relatively high and stable coral cover in percentages greater than the Caribbean regional average of 20% (TNC 2006). Overall hard coral percentage cover recorded from Reef Check surveys for the period 2004 to 2007 ranged between 2.2% and 37.5%.

The trend of algal dominated reefs is still visible on several reef systems across the island. This continued proliferation is due to the continued nutrient input and the absence of grazers. Overfishing has removed virtually all large predatory species, like sharks, snappers and groupers, and has sharply reduced the size of herbivores, namely parrotfish and surgeon fish. Notwithstanding of the myriad of environmental and biological factors affecting the reefs some have been able to maintain a relatively stable coral cover. Over the period 2001 to 2007 four sites (Ireland Pen, Bloody Bay, El Punto Negrilo and Little Bay) within the Negril Marine Park have been continuously monitored (Fig. 3). They have maintained overall coral percentages of 17.5%, 9.9%, 16.0% and 17.4% respectively.

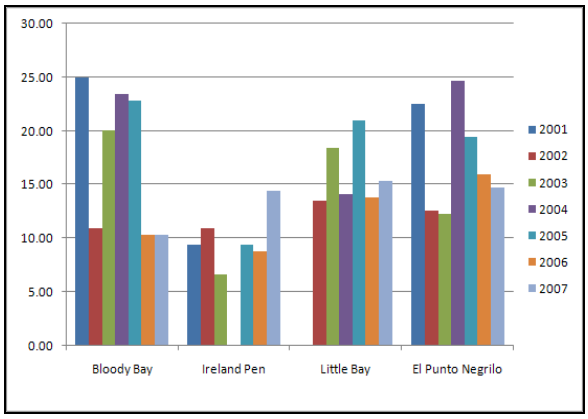

Figure 3 – Hard coral percentage cover on long-term monitoring sites in Negril, Jamaica: 2001 -2007

The region is now experiencing a new threat namely the Indo-Pacific lionfish (*Pterois volitans*). It has been reported in all the countries of the node except Haiti; this absence may however be due to a lack of monitoring. The presence of this invasive has the potential to further damage the already threatened fish population of the region and also affect coral health. This organism may also pose a threat to the highest economic earner of the Caribbean; tourism. In Jamaica they have been sighted on tourist dive sites and have also been encountered in fish pots at depths up to 60 feet.

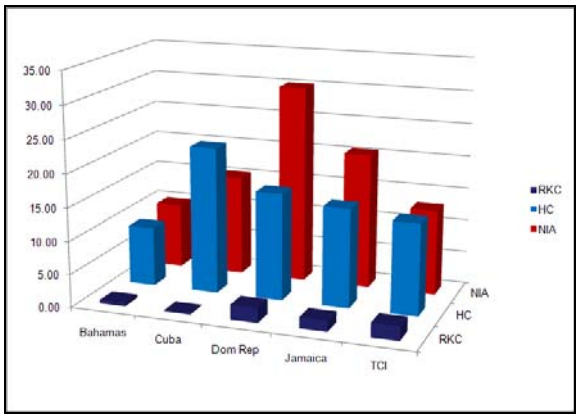

Figure 4 – Reef Check data 2004 – 2007showing average percentage cover for recently killed coral, hard coral and nutrient indicating algae

Discussion and Conclusion

The reef ecosystems of the region are comparable with respect to challenges encountered, coral cover, species distribution and composition (Figs. 1 and 4). While some reefs are indeed in serious trouble, others remain healthy and appear to be developing some resilience to the effects of natural forces and human activity. These reef systems appear to be able to recover from stress events such as high water temperatures, intense storms and prolonged sedimentation. This is evident on several Jamaican reef sites such as Little Bay, Westmoreland and Columbus Park, St. Ann which are sediment impacted sites. The degree of recovery is however dependent on time-span between stress events.

Hope for the reefs is also evident in the recovery of *Diadema antillarum* densities on some reefs along with *Acropora* sp. recruits. Current fishing practices however need to be changed to aid in the recovery.

In several countries, active monitoring is still not a standard government tool to manage the ecosystem. Governments need to develop strategies and methodologies for the assessment of the coastal area as a base for sustainable development. They also need to implement programmes and regulations to enhance coral resilience.

The region also needs to collaborate and formulate measures to combat the effects of the common problem of climate change and ocean acidification. A programme to deal with the lionfish invasion is also critically needed.

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New insights into the exposure and sensitivity of coral reefs to ocean warming

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Abstract. Increases in coral bleaching events over the past 20 years are correlated with increases in sea surface temperature (SST). While SSTs have increased everywhere in the tropics, and coral bleaching events have occurred in all coral reef provinces, the frequency and intensity of coral bleaching events have varied considerably from region to region. Unless these patterns are random, we can derive useful information about why some reefs have been less vulnerable to increasing SST, and use this information to guide conservation efforts. Coral reef susceptibility to bleaching is a function of exposure to some stress (elevated SST) and sensitivity to that stress. To better characterize differences in coral reef vulnerability to increases in SST, we analyzed weekly SST records with coral bleaching records for reefs at the global scale. Our results indicate that variability of maximum SSTs is an additional factor that determines reef sensitivity to temperature increases, and taking this into account allows better predictions of coral bleaching in regions with low temperature variability.

Key words: bleaching, thermostat, SST, temperature, vulnerability

Introduction

The most commonly used predictor of when accumulated heat stress will cause coral bleaching is the degree heating week (DHW; (Liu et al. 2006)). The DHW index combines the severity of thermal stress (SST > 1ºC above the climatological maximum) with its duration over a rolling 12-week period. The NOAA Coral Reef Watch Program developed and maintains a very useful real-time bleaching predictive tool that calculates the DHW for reef regions worldwide. These calculations are based on twice-weekly satellite-derived SSTs at a spatial resolution of 50 km. DHW's are commonly used in studies examining the causal relationship between rising sea surface temperature (SST) and coral bleaching, but the method is known to over-predict and under-predict bleaching in some areas (e.g. McClanahan et al. 2007b) due to a range of factors associated with the scale and use of satellite data, to biological traits such as species composition.

When working with either historical or predicted sea surface temperature data, the temporal and spatial resolutions are much coarser; usually the data are weekly to monthly, and at best are gridded at 1x1° resolution. Previous studies that have used global climate model output to predict future bleaching frequency have used either a monthly SST threshold of 1ºC above normal maximum to predict bleaching (Hoegh-Guldberg 1999) or a degree heating month (DHM) index (Donner et al. 2005).

In order to develop a more generalized predictive tool for coral bleaching – one that can be used with more coarsely resolved past SSTs or model-projected SSTs, we examined ways to improve the DHW index toward predictions of coral bleaching. Our examination focuses on the concept of susceptibility, that is, not only a coral's exposure to high temperature event, but also its sensitivity to that exposure.

While it is clear that coral sensitivity to some maximum temperature varies regionally with differences in the climatological maximum, several studies have also suggested that corals from high-variability environments are more temperature-tolerant than those from low-variability environments (McClanahan et al. 2007a; Kleypas et al. 2008). In a rather crude analysis, (Kleypas 2006) found that the threshold of 1ºC above climatological maximum under-predicted coral bleaching in regions where the maxima varied little from year to year (e.g., in the eastern equatorial Pacific; Fig. 1), and that predictions improved if the threshold considered variability of maximum SST (Fig. 2). Here, we more closely examine whether coral sensitivity to elevated temperatures varies as a function of temperature variability.

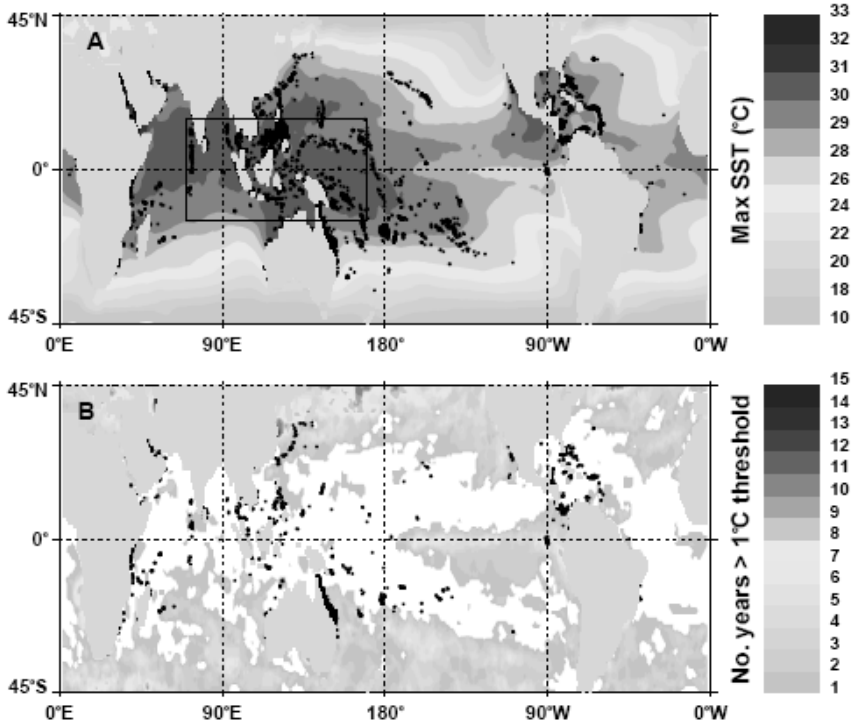

Figure 1: (A) Average annual maximum sea surface temperatures contoured showing locations of all coral reefs. (B) Number of years that the SST maximum exceeded the climatological maximum + 1ºC for at least one week. Black dots indicate reefs that have experienced bleaching (from Kleypas 2006).

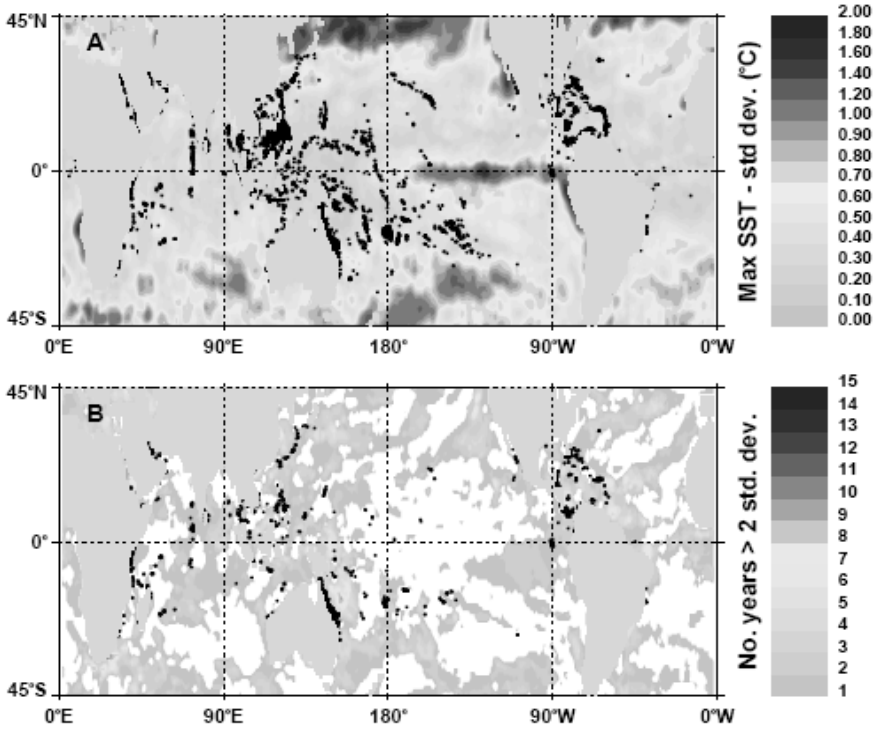

Figure 2: (A) Standard deviation of the average annual maximum sea surface temperature, showing locations of all coral reefs. (B) Number of years that the maximum exceeded the climatological maximum + 2 standard deviations for at least one week. Black dots indicate reefs that have experienced bleaching (from Kleypas 2006).

Methods

Data. Coral bleaching data were obtained from the ReefBase Global Bleaching Database (ReefBase-Project 2007). This data set is a compilation of mostly volunteer reporting of observations. NOAA's Coral Reef Watch Program has facilitated the ability to verify and qualify bleaching in areas where temperatures have exceeded certain thresholds, but coral bleaching has almost certainly been under-reported. The bleaching reports were summarized for each 1x1° cell, yielding a total of 516 unique reef locations that have experienced at least one bleaching event of any severity between 1982 and 2006.

SST data were obtained from the weekly Reynolds Optimally Interpolated SST (OISST) data set (Reynolds et al. 2002) for the years 1982–2006. The OISST has a spatial resolution of 1°x1°, and incorporates in situ observations as well as satellite-based observations.

Analysis. Using the weekly OISST data, the climatalogical maximum SST was determined for each grid cell as the average maximum for the first ten years of the OISST data (1982–1991). The standard DHW index (DHW_1), which uses an absolute threshold (1ºC above climatological maximum), and a modified DHW index (DHW_2) in which the threshold bleaching temperature is based on natural variability (for example, when SST > 2 standard deviations (SD) above climatological maximum), were then calculated for the entire OISST record. For DHW_2, we considered the variability of the annual maxima rather than that of the entire SST record. For both indices, we assumed that a bleaching event was possible once the DHW exceeded a designated threshold.

To account for the fact that bleaching is often observed some time after a temperature excursion, we assumed that a bleaching observation reported within 3 months following a predicted bleaching event was part of that event, and multiple observations within that 3-month time period were consolidated as one event. This was determined as the optimal time period for capturing most bleaching observations following a temperature maximum. Thus, a total of 725 bleaching events were identified for the 516 grid cell locations.

We determined "best fit" by maximizing the number of correct bleaching predictions (bleaching predicted and observed), while minimizing false negative (Type II error; bleaching not predicted but observed) and false positive predictions (Type I error; bleaching predicted but not observed). False positive predictions are particularly difficult to evaluate in this analysis because of the incomplete nature of the ReefBase data; that is, we do not have a good estimate of the number of bleaching events that occurred but were not observed or reported.

Results

Applying the standard bleaching index (DHW_1) with the weekly OISST data predicted 159 bleaching events over the 25-year period, but fails to predict 566 bleaching events (1.0ºC above maximum; Table 1). The number of predicted bleaching events that were not observed (438) is more than twice the number of correctly predicted bleaching events. If one assumes that, when bleaching occurred within a grid cell, at least one report was made and included in the ReefBase dataset, then the DHW_1 method correctly predicted bleaching 14% of the time [% correct = number of correct predictions/(total number of correct + incorrect predictions)]. If the temperature threshold is lowered to 0.7°C, then the method correctly predicts bleaching 19% of the time.

Table 1: Comparison of DHW_1 versus DHW_2 predictions of coral bleaching. Bold rows are predictions using the standard threshold (DHW_1), and the best results when using standard deviation of the annual monthly maximum (DHW_2).

DHW_1 ºC above climatological maximum	Correct	Incorrect		% correct	DHW_2 SD above climatological maximum	Correct	Incorrect		% correct
	Predicted and Observed	Type II Not Predicted but Observed	Type I Predicted but not Observed			Predicted and Observed	Type II Not Predicted but Observed	Type I Predicted but not Observed	
0.5	452	273	2048	16.3	1.5	434	291	1673	18.1
0.6	397	328	1490	17.9	1.6	407	318	1465	18.6
0.7	338	387	1069	18.8	1.7	382	343	1247	19.4
0.8	273	452	778	18.2	1.8	356	369	1087	19.6
0.9	210	515	578	16.1	1.9	326	399	926	19.7
1.0	**159**	**566**	**438**	**13.7**	**2.0**	**308**	**417**	**788**	**20.4**
1.1	107	618	303	10.4	2.1	276	449	670	19.8
1.2	69	656	213	7.4	2.2	262	463	583	20.0
1.3	54	671	161	6.1	2.3	236	489	492	19.4
1.4	43	682	111	5.1	2.4	212	513	423	18.5
1.5	35	690	81	4.3	2.5	189	536	374	17.2

DHW_2 performed best (20.4% correct predictions) using a 2.0 standard deviation threshold. 308 bleaching events over the 25-year period were correctly predicted, 417 bleaching were not predicted, and 788 events were predicted but not observed (Table 1). The % correct predictions dropped off at higher and lower standard deviation thresholds.

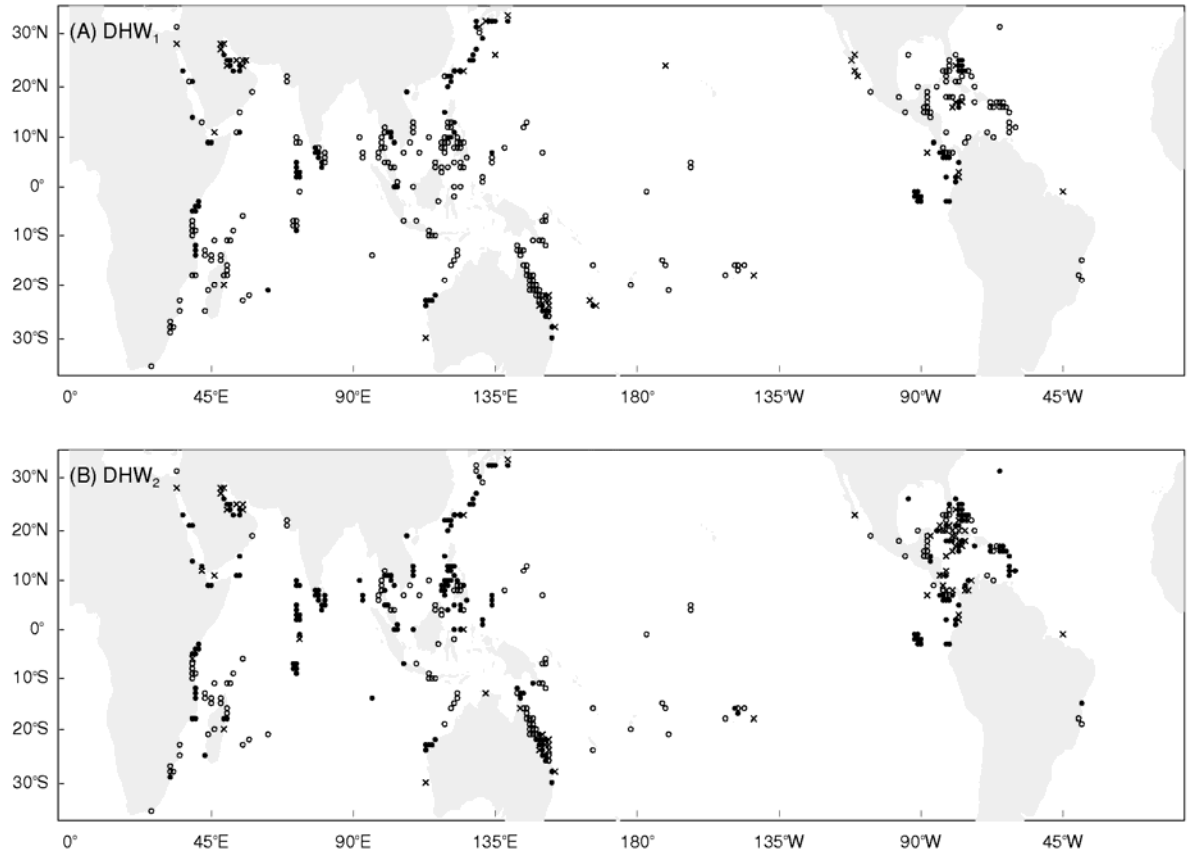

Figure 3: (A) DHW_1 predictions for 1997–1998. Solid black circles indicate a match: bleaching was observed where it was predicted. White circles indicate where bleaching was observed but not predicted (Type II error). The X's show locations where bleaching was predicted but not observed (Type I error). (B) DHW_2 predictions for 1997–1998.

Discussion and Conclusions

This rather simple analysis illustrates that neither method predicts bleaching well, but it does suggest that natural variability of the annual maximum SST is a factor that determines regional thresholds to bleaching. The most obvious error in both methods is the failure to predict bleaching when it occurs (Type II). The DHW_2 method seems to do a slightly better job at predicting the observed bleaching events. Both methods suffer from high Type I errors, such that bleaching is predicted much more often than it is observed. This over-prediction can occur for two reasons: 1) the coral reef communities did not bleach (a true Type I error); or 2) at least some bleaching events were not reported (and indeed, were probably not even observed) and thus were not included in the ReefBase data set. Thus, the number of over-predicted reefs is almost certainly less than shown here.

The distribution patterns of the correctly and incorrectly predicted bleaching events reveal a potential bias in the DHW_1 method in that it does a better job in high variability regions such as at higher latitudes and in upwelling regions (compare Figs. 1 and 2 with Fig. 3). The DHW_2 method seems to do a better job across regions, and successfully predicts bleaching events in regions with low SST variability (e.g. equatorial regions; Fig. 3). This implies that current bleaching predictions underestimate bleaching in low variability regions, such as in the western Pacific warm pool. It also implies that the conditioning of corals to bleaching is not only a function of the maximum temperatures that they are accustomed to, but also the variability in those maximum temperatures. Fig. 4 presents a clustering of coral reefs based on SST minima, maxima, and variability. We propose that these reef clusters have different thresholds to changes in temperature

extremes and variability, and that bleaching indices should take into account these differences. Finally, many other factors besides SST affect the bleaching thresholds in corals, such as solar radiation (Brown, Dunne 2008), mixing (Nakamura, Yamasaki 2005; Fabricius 2006), community structure (McClanahan et al. 2005), and clade-type of the zooxanthellae symbionts (Baker 2003; Berkelmans, van Oppen 2006; Jones et al. 2008). Like SST patterns, these variables also vary from region to region, and many have been shown to be important factors that contribute to regional variations in vulnerability to coral bleaching (McClanahan et al. 2007a; Maina et al. 2008).

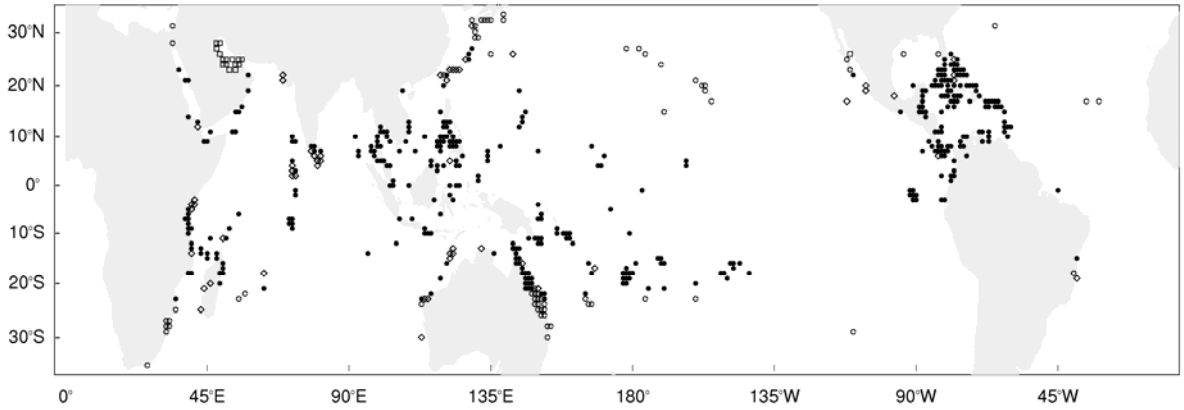

Figure 4: Reefs with common temperature characteristics, based on the minimum, maximum and variance of SSTs from the weekly OISST data (Reynolds et al. 2002).

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Status of Coral Reefs in Post-Tsunami Period in Andaman & Nicobar Islands

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Abstract Andaman & Nicobar Islands, located in the Bay of Bengal off the eastern coast of India, are one of India's four major sites with coral reefs, mostly fringing types. The survey carried out by the UNDP team along with experts from India in 2001 recorded 197 species of corals in the Andaman group against 179 species reported earlier by Pillai (1983) for the entire Andaman & Nicobar Islands. The survey done by Kulkarni and Saxena (2002) for 24 coral reef sites identified under a coral reef monitoring action plan showed the distribution, status of health and mortality of corals. The Tsunami that struck these islands on 26th December 2004 led to vast destruction of coral reefs mainly due to geo-morphological changes resulting in uplifting and exposure of reefs in the northern islands and submergence in the southern islands A rapid assessment done by the NGO Reef Watch in 2005 showed that there was overall 20% mortality in Andaman group of Islands while in Nicobar group of Islands, up to 80% mortality was observed. The present study is based on the survey being carried out at different coral reef sites to monitor the recovery processes. The initial results show not only recovery but also changes in the distribution pattern of corals.

Key words: Tsunami, Live coral cover, Fringing Reefs, Soft Corals, Sedimentation

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Introduction

The Andaman and Nicobar Islands (A & N Islands) constitute one of the hot spots of the biodiversity in India and exhibit a variety of ecosystems such as tropical forests, mangroves and coral reefs. They consist of about 570 islands of varying sizes and are the largest archipelago system in the Bay of Bengal. They are located latitudinally between 06º 45' N to 13º 41' N and longitudinally between 92º 12' E to 93º 57' E. Their rotal geographic area is 8249 km^2 and the length of coastline is 1962 km. Islands north of 10º N latitude belong to Andaman group of Islands and those south of 10º N latitude fall in Nicobar group of Islands. Only 38 islands are inhabited of which 12 belong to Nicobar group (total islands = 24).

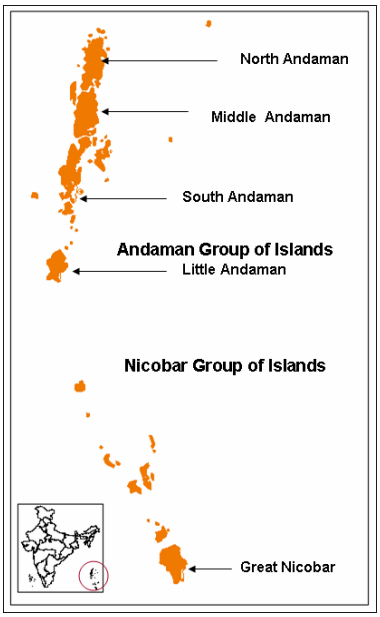

Fig. 1: Location map

Coral Reefs in A & N Islands

Coral reefs of the A & N Islands are highly diverse and reefs cover about 2000 km^2 i.e., 6% of the total

continental shelf of these islands, mainly as fringing reefs. A barrier reef of about 320 km length with 4 m deep lagoon has been reported on the western side of South and Middle Andaman Islands. It is separated by an 80 m deep channel. The common coral genera contributing to the reef formation in these islands are *Acropora, Montipora, Pocillpora, Porites, Gonopra, Favia, Echinopora, Fungia, Milleporina, Hliopora* etc.

Pre-tsunami status

The A & N Islands have the richest coral fauna among the four major coral reef sites of India (the other three sites being the Lakhsadweep, Gulf of Mannar and Gulf of Kutch). Alcock (1893) during his visit to Port Blair in 1888 gave a brief description of reefs here and also pointed out the adverse effect of silting in the inshore waters on coral growth. Sewell (1922) described the morphology, community ecology and formation of the reefs of Nicobar. Scheer studied reefs of Great Nicobar and Tillongchang islands. Scheer and Pillai (1974) gave an account of corals of Nicobar Iislands. Pillai (1983) reported 135 species belonging to 59 genera and Tikedar et al., (1986) reported 179 species belonging to 61 genera. A recent survey by a UNDP-GOI team who covered selected sites in the Andaman group recorded 197 species of stony corals and estimated the total number of stony coral species to be 234 in Andaman group of islands only (Turner et. al. 2001). Kulkarni and Saxena (2002) studied the status of coral reefs at 24 sites distributed over both groups of Islands under the programme of the Indian Coral Reef Monitoring Network (ICRMN). Wafar (1986) estimated the reef area of the Andaman Islands to be 11,000 km2 and that of Nicobar Islands to be 2,700 km^2. Nayak et al. (1994) estimated the total reef area to be 953.3 km^2 using remote sensing. Later, Turner et. al. (2001) estimated the reef area of Andaman Islands using remote sensing data, to be 11,989 km^2.

Post-tsunami studies

The Tsunami that struck these islands following a massive earthquake on 26th December 2004 caused heavy destruction not only of life and property but also of ecological resources. Reefs were damaged extensively both in Nicobar group as well as in northern islands of Andaman group. No detailed study on impact of Tsunami was carried out, only a few rapid assessments of damage to reefs. The study based on remote sensing data carried out in 2005 by the Space Application Centre (SAC), Department of Space, revealed that the tsunami caused two main types of damage to reefs- (i) total erosion or breaking up of reefs, and (ii) deposition of sand, mud and detritus on reefs. The report further revealed that the total loss (erosion) of coral reefs in the Andaman Islands was 22978 ha and in the Nicobar Islands it was 17180 ha. Reef Watch Marine Conservation made a rapid survey of selected islands in 2006 and reported that reefs in the North and Middle Andamans suffered mainly because of uplifting of land and exposure of reefs. The damage was less severe (about 20%) in reefs around South Andamans, but it was heavy in Nicobar group of Islands. The present paper deals with the assessments being done in details on selected representative sites in major island groups of A & N Islands.

Methods

The survey was carried out for Northern group of Andaman Islands in the year 2006 and for Southern Andaman and Nicobar group of Islands in 2008. The survey methods used included LIT (Linter Intercept Transect), Manto Tow and species diversity and area estimation in exposed reefs.

Results and discussion

The damage to coral reefs took place as a result of two events that occurred on 26th December 2004- the first one being the massive earthquake with intensity of 9.3 Richter scale causing geo-morphological changes in these Islands. The second was in the form of a giant tsunami wave. The earthquake resulted into uplifting of land by more than 1 m in the North and Middle Andamans and subsidence of land by more than 1 m in the Nicobar group of Islands. This led to exposure of reefs in the northern Islands of the Andamans where mortality ensued due to exposure of reefs while in the Nicobar group of Islands the damage was due to wave action. Many corals were uprooted, broken and turned upside down. Moreover, a lot of sediments was mobilized by wave action and currents, leading to smothering of corals.

North Andamans

The islands studied included Landfall, East, Smith & Ross, Aves, North Reef and Interview Islands. Significant mortality was observed in all the aforementioned Islands except in Avis Island. Following is the Island-wise account:

Landfall Island: Most reefs of Islands were totally exposed. Dead corals constituted of about 85 % of the reef area. *Acropora* constituted 65 % of the coral diversity, the species recorded included *A. florida,*

A. cytheria, A. monticulosa, A. humilis, A. palythoa, A. palifera and A. hyacinthus. Massive and sub-massive corals constituted about 20% and major genera were *Heliopora, Pocillopora verrucosa, P. eudoxyi, Fungia, Goniastrea, Favites, Porites lutea* and *Montipora.*

Fig. 2: Landfall Islands- exposed reefs

East Island: Like at Landfall Island, most of the corals were exposed and dead. Mortality was above 70 % and *Acropora* constituted 50 % of species. Massive and sub-massive corals constituted 20 %. Eight *Acropora* species (same as at Landfall Island), *Platygyra pini, Ctinactis echinata, Hydnopora rigida, Pocillopora damicornis, P. verucosa, Pocillopora euidui, Hydnopora macroconus, Symphyllia radians and Porites lichen.* were recorded.

Fig. 3: East Island

Smith & Ross Islands: Pre-Tsunami assessment by Kulkarni and Saxena (2002) showed live coral cover of 54 % to have declined to 3 %. Earlier dominant species were *Acropor*a (45%), *Porites* (16%) and *Montipora* (9%). After the tsunamic, *Porites* (49%) dominated followed by *Favites abtidia* (21%) and *Pavona* (18%).

Aves Island: Not much damage was recorded at this island. Pre-tsunami records showed live coral cover to be 62% and a slight decline to 50%. Earlier *Acropora* constituted 40% followed by *Porites* (23 %). After the tsunami, *Acropora* constituted 83 % and *Porites* 16 % of the community.

North Reef: This was one of the best reef sites of the Andaman Islands. Reefs suffered here heavily due to uplifting and exposure of submerged land. Live coral cover which was 74% earlier declined to 13%. *Acropora* dominated the live coral (77 %).

Interview Island: Reefs were exposed. Pre-tsunami live coral cover was 68%. post-tsunami the dead coral cover was 80%. Massive and sub-massive coral dominated (70%) and 42 species were recorded. *Diploastrea heliopora* dominates the sub-massive species.

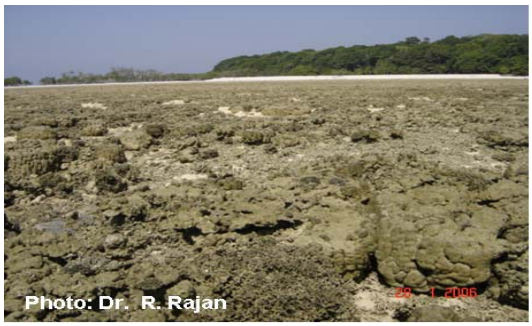

Fig. 4: Interview Island

South Andamans

In the South Andamans, seven Islands of the Mahatma Gandhi Marine National Park were studied which included Alexendra, Belle, Baot, Chester, Grub, Snob and Redskin.

Fig. 5: Mahatma Gandhi Marine National Park

Details of status of coral reefs in pre and post tsunami period are given in Table 1.

Table 1: Status of coral reefs in MGMNP

Island	Present Status	% Live Coral	
		Pre-Tsunami	Post Tsunami
Alexendra	Degraded, mostly Boulders	30 %	10-15 %
Bellie	Highly degraded, Massive corals	N. A.	< 10 %
Boat	Highly degraded, Massive corals	16 %	<10 %
Chester	Degraded, mostly Boulder	N. A.	40 %
Grub	Healthy, mostly branching	42 %	Around 60%
Redskin	Degraded, Mostly boulders, soft corals also present	33 %	20 %
Snob	Highly degraded, Massive corals	N. A.	20 %

Al though the tsunami did affect the reefs, the effect was not very pronounced. The reasons for degradation of reefs in this Marine National Park are mostly local factors that include high sedimentation rate, turbidity and oceanic currents. Corals were in good health around Grub and Chester but not at other islands.

Nicobar Islands

Heavy destruction, but also recovery was observed. Impacts were caused by strong tsunami waves as these islands were very near to the epicenter of the quake, as well as the heavy siltation caused due to oceanic currents.

Car Nicobar: High mortality (70 %). Live coral only 10 % while in 2003 live coral cover was above 70 %. Some reefs were totally destroyed up to a depth of 20 m. Some patches in shallow waters had survived.

Teressa: Less damage with dead corals at 37 %. Live coral cover was 34% and soft corals constituted 14.3%. *Montipora* was the most common genus (21 %), followed by *Porites* (6 %) and *Pocillopora* (6 %). Other species included *Acropora and Goniastrea.*

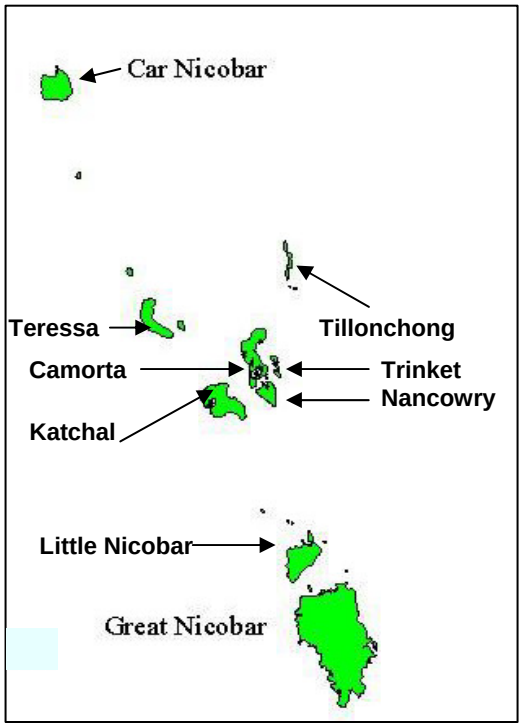

Fig. 6: Nicobar Islands

Camorta: Heavy damage. Dead corals extended over 80% of reef area, live coral on 7 %. Soft corals formed 4 % of total live corals. *Favites* was the most common genus (3 %), followed by *Porites* (2 %), *Siderastrea, Platygyra and Favia.*

Katchal: Signs of recovery. Dead coral covered 49 % of the reef area. Live coral cover 24 % and of this soft corals 12 %. Among live corals, *Heliopora* dominated with 22 %. Other genera include *Montipora, Pocillopora, Fungia and Symphylli.*

Trinket: Dead corals covered 62 % of the reef area and live corals only13 %. Soft corals only in some pockets. *Platygyra* was the most common genus (4.5 %), followed by *Acropora (4* %) and *Porites* (2 %). Other present genera included *Montipora, Goniastrea, Pocillopora and Heliopra.*

Nancowry: better live coral cover (39%) of which 10% was soft coral. Dead coral cover only 13%. Of the live corals, *Pocillopora* dominated with 12 %, followed by *Porites* (6%), *Echinopora* (5 %) and *Favites* (4.5%).

Little Nicobar: High mortality due to sedimentation and wave action. Live coral cover less than 10%.

Great Nicobar: More than 70 % of corals dead. Maximum damage (80 %) due to direct impact of Tsunami and 20 % damage due to sedimentation. Prior to Tsunami the live coral cover as assessed in 2003 was about 70%, post-tsunami is had declined to 20 %.

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Invertebrates, Communities, and Reef Health in Airai, The Republic of Palau

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Abstract
During 2006 to 2008, a women's group studied the distribution, densities, sizes and harvest rates of edible invertebrates in southeast Babeldaob. Replicate transect lines, mobile GPS tracking units and traditional harvesting methods were used. Women covered a mean area of 2,520 m^2h^{-1} or 0.25ha h^{-1}. The mean CPUE for weight for all invertebrates collected was 2.4 kg $w^{-1}h^{-1}$ and the mean CPUE for weight for all invertebrates per hectare was 6.2 kg $w^{-1}h^{-1}ha^{-1}$ and differed significantly between sites. Low densities of *Hippopus hippopus* and *Holothuria scabra* were found. Low densities of *Tripneustes gratilla* may indicate a potential collapse in this fishery. Spawning of wild *Tripneustes gratilla* was observed in April 2007. Small and patchy populations were measured at finer spatial and temporal scales with input from resource users to better estimate and detect change in populations. One market sold a mean weight 1345 kg of selected invertebrates between 2000 and 2007. Airai State conservation officers are working with research, enforcement and community partners to manage these resources. Key invertebrates are potential indicators of reef health. These results can be used to set size limits, threshold densities of specific species and seasonal and site closures for specific sites.

Key words: Invertebrates, women, harvest, effort, management.

Introduction

Overfishing, pollution, habitat loss and global warming threaten fisheries worldwide. *Tridacna* clams are on the UN's red list of endangered species (IUCN 1983). *H. hippopus* is sensitive to elevated seawater temperature; mass mortality occurred in the Philippines during the 1998 ENSO event (Blidberg et al. 2000). *Holothuria scabra* is one of several prized sea cucumbers used as food, medicine and as an aphrodisiac. All *Tridacna* species and the sea cucumbers, *H. scabra* and *Actinopyga miliaris* are banned for export in Palau; *Tridacna* spp. are produced in hatcheries in Palau and the region (Heslinga et al. 1990). The gonads of *Tripneustes gratilla* were over harvested and the fishery collapsed in the Philippines (Juninio-Meñez et al. 1998). *Stichopus vastus*, an edible sea cucumber with regenerative properties and *Lambis lambis*, the culturally significant spider shell are declining in numbers. Women play a critical role in nearshore fisheries (Matthews and Oiterong 1991, Lambeth 1999). Our objectives were to work with a women's group in Airai to determine relative abundance, distribution, size and catch efforts for populations of key invertebrate species; share the results in the community; and discuss management options.

Materials and Methods

The Republic of Palau is the westernmost archipelago of the Caroline Islands in Micronesia that lies within latitudes 8°12' to 2°48' N and longitudes 131°07' to 134°44' E The study site is located in Airai State in southeast Babeldaob, the largest island in Palau (Fig.1).

Figure 1: Study sites (West, South, East) in Airai State, Southeast Babeldaob, Republic of Palau (Source: PALARIS).

Three sites along the West, South and East coasts of Airai (Fig. 1) were surveyed over a 34 day period from November 2006 to April 2008. At least three 50m transects were set 100m apart and parallel to the reef covered a minimal area of 750m^2 at each site for the key invertebrates optimizing overlapping habitats. Seagrass density and canopy height was measured within 0.25m^2 quadrants at either 4m, 5m or 10m intervals along the transect line; invertebrates were quantified within 5m of the transect line. The line intercept technique was used to quantify coral cover at the West site. Invertebrates were counted, measured to the nearest 0.1cm, and all but the unharvested embedded clams were weighed to at least the nearest 0.1 kg. The wet weight of harvested clam meat and the shell length were measured. Weight and lengths for *Tridacna crocea* and *T. maxima* where combined as embedded clams for this study as women did not distinguish between them in the field. Sea cucumber measurements are complicated because they change shape and retain water and sediment (Preston 1993). In this study, sea cucumbers were weighed and measured after sand and excess water was removed and if contracted, they were left few minutes before length was measured. The taxonomy of the Genus *Actinopyga* is under revision: the local name "cheremrum" represents several species. However we are referring to the smallest species in this genus with a variable coloration. The total weight of sea urchins was measured because they are sold unprocessed. Photo-documentation was conducted during the study.

The area covered during traditional harvesting was quantified using an "in-water" tracking device designed by Coral Reef Research Foundation which was a modified PVC waterproof casing that can a small GPS unit can be inserted and sealed with an screw top, airtight lid. An extended PVC pipe is attached to the device that has a foam float for buoyancy. A Garmin GPS unit was set to track position at 15 sec intervals. The device was either tied onto a person or a collecting basin. Data was downloaded, analyzed and mapped using ©Arcview software. Women searched a mean width of 4m and harvested singly or in groups. Individual and group catches were quantified. The non-parametric Kruskal-Wallis Test was used to test the null hypothesis that the CPUE for numbers (ct $w^{-1}h^{-1}$), the CPUE for weight (kg $w^{-1}h^{-1}$), the CPUE for weight per hectare (kg $w^{-1}h^{-1}ha^{-1}$), size and densities, did not differ significantly between the 3 sites. The Rank Sum Test was used to compare 2 sites (Ambrose, Ambrose 1987). Results were presented to over 250 people in the Airai community in a series of power point presentations. Concerns and comments were recorded and summarized.

Results

Seagrass Density and Substrate Cover

A total of 189 0.25m^2 quadrants were placed along transects at the West (n=72) South (n=73) and East (n=44) sites. The mean density for all seagrass species (plants 0.25/m^2) at each site was as follows: East: 3.1 (sd=4.4); South: 1.3 (sd=2.8) and West: 1.1 (sd=2.1). For all seagrass species, the mean height (cm) at each site was as follows: West: 13.9 (sd=12.3, n=134); South: 12.4 (sd=11.2, n=262); East: 9.2 (sd=5.3, n=381). The mean densities (plant 0.25/m^2) for each species at each site were as follows: West: *E. acoroides* 1.5 (sd=1.9), *T. hemprichii* 2.7 (sd=2.9); *C. rotundata* 0.3, sd=1.1); South: *E. acoroides* 1.4 (sd=1.7), *T. hemprichii* 3.2 (sd=4.6); *C. rotundata* 6.4 (sd=6.1); and *Syringodium isoetifolium* 0.2 (sd=0.8); East: *Enhalus acoroides* 4.1 (sd=3.2); *Thalassia hemprichii* 1.5 (sd=2.2); *Cymodacea rotundata* (6.4, sd=6.1); and *S. isoetifolium* 0.2 (sd=0.8). Substrate cover at the West site along 4 transects included live coral 54.6% (sd=36.1), dead coral 6.4% (sd=2.8), coral rubble 15.0(sd=7.0) and sand 25.8% (sd=6.2). An estimated 36% of the live coral was of the genus *Porites. T. crocea* and *T. maxima* were found in habitats with high *Porites* sp. coral cover.

Track Paths

The mean speed obtained from the mobile GPS units was 630m w^{-1} h^{-1} (sd=198, n=24). Based upon a mean search width of 4m, women covered a mean area of 2,520m^2h^{-1} or 0.25ha h^{-1}. When in deeper water, women swam 91% of the time (sd=6%, n=13) and stopped 9% (sd=6%, n=13). When in shallow or exposed reef areas women walked 70% of the time (sd=11%, n=9) and stopped 30% of the time (sd=11%, n=9). Women stopped to either harvest, process, or rest; they swam or walked to either search or reach a harvest area.

Total Biomass and Catch per Unit Effort

The mean CPUE for weight for all invertebrates collected was 2.4 kg $w^{-1}h^{-1}$ and the mean CPUE for weight for all invertebrates per hectare was 6.2 kg $w^{-1}h^{-1}ha^{-1}$. The CPUE in total wet weight for all invertebrates collected (H=10.14) and the CPUE for total wet weight harvested per hour per hectare (H=14) significantly differed between sites ($X^2_{.05[2]}$ value=5.99) as shown in Table 1.

Table 1. Catch per Unit Effort (CPUE) in wet weight (kg $w^{-1}h^{-1}$) and CPUE of wet weight per hectare (kg $w^{-1}h^{-1}ha^{-1}$) for all invertebrates (sd=1) and the number of samples (n).

Site	kg $w^{-1}h^{-1}$	kg $w^{-1}h^{-1}ha^{-1}$
West	2.1 (2.0) n=10	7.3 (13) n=10
South	2.4 (2.2) n=12	6.9 (9.8) n=12
East	2.8 (2.2) n= 6	4.3 (4.9) n= 6
Mean	2.4 (2.0) n=28	6.2 (9.8) n=28

Tridacna crocea and *Tridacna maxima*

The mean clam size for *Tridacna crocea* and *Tridacna maxima* combined for all transects was 7.0cm (sd=3.5, n=208). Women harvested clams ranging from 6.5 to 15cm with a mean size of 10.2cm (sd=1.8, n=130). For all sites, the mean CPUE for the numbers of *T. crocea* and *T. maxima* was 11.8 (sd=11.9, n=37) and the mean CPUE for weight was 0.62kg (sd=0.51, n=32). The CPUE significantly differed between sites for numbers (H=42) and weight (H=204) with an $X^2_{.05[2]}$ value of 5.99 (Table 2). The mean density of clams was 0.07(sd=0.06, n=14) compared to 0.03(sd=0.05, n=78) in 2003. Clam densities, harvest sizes and sizes along the transects showed no significant difference between sites. The percent of clams that were less than the minimum harvestable size (>6.5cm) at each site were as follows: West: 11%, South: 42% and East: 50%.

Table 2. The mean CPUE for counts (ct $w^{-1}h^{-1}$) and weight (kg $w^{-1}h^{-1}$) for *Tridacna crocea* and *Tridacna maxima* (sd=1) and sample number (n). $X^2_{.05[2]}$ value = 5.99.

Site	ct $w^{-1}h^{-1}$	kg $w^{-1}h^{-1}$
West	17.7 (14.4) n=18	0.66 (0.52) n=18
South	5.8 (3.7) n=12	0.26 (0.15) n=10
East	7.1(6.5) n=7	0.15 (0.11) n=4
Mean	11.8 (11.9) n=37	0.62 (0.51) n=32
H	*42	*204

Hippopus hippopus and *Tridacna squamosa*

The mean size for all harvested *H. hippopus* was 22.6cm (sd=6.4, n=43) ranging from 8cm to 45cm. The mean CPUE for numbers of *H. hippopus* was 1.7 (sd=1.45, n=35). The mean CPUE for weight was 1.0kg (sd=0.9, n=26). The mean CPUE for the numbers and the mean CPUE for weight and mean size of harvested *H. hippopus* were significantly different between sites. (The H statistic was 28, 27.6 and 56 respectively; all values were greater than the $X^2_{.05[2]}$ value of 5.99). Mean sizes for each site were as follows: West: 23.3cm (sd=12.7, n=3); South: 22.6cm (sd=6.4, n=43) East: 31.7cm (sd=16.2, n=10). Mean CPUE for counts for each site were as follows: West: 0.83 (sd=0.59, n=6.3); South: 1.81(sd=1.5, n=17) East: 2.16(sd=1.86, n=11). Mean CPUE for weight for each site were as follows: West: 0.76kg (sd=0.79, n=6); South: 0.82kg (sd=0.65, n=11) East: 1.29kg (sd=1.14, n=9). The mean density of *H. hippopus* was 0.011/m^2 (sd=0.007, n=5) compared to 0.002/m^2 (sd=0, n=7) in 2003. The CPUE for *T. squamosa* at the South site was 3.5/w/h.

Tripneustes gratilla

The mean size for all harvested *T. gratilla* was 6.3cm (sd=1.0, n=233) ranging from 4.3 to 11cm. The mean CPUE for the number was 34 (sd=20, n=11). The mean CPUE for weight was 5.6kg (sd=1.3, n=11). The mean CPUE for number and weight significantly differed between the South and West sites (T =18) with a critical value for Rank Sum Test =20 at α=0.05). The East site is not a known site for *T. gratilla*: only dead tests and a few urchins were found. At the South site the mean CPUE for numbers was 47 (sd=13, n=4) during November 2006 and 91 (sd=63, n=3) during March 2007 which was the highest CPUE recorded in this study; the mean CPUE for weight was 4.7kg (sd=1.0, n=5) in November and 18 kg (sd=9.5, n=3) in March; the mean diameter was 6.2cm (sd=0.85, n=95) in November and 6.0 cm (sd=0.54, n=109) in March. During April 15, 2007, *T. gratilla* were observed spawning in the seagrass at 4pm at the South site. At the West site, the mean CPUE for numbers was 27.6 (sd=9.3, n=6), the mean CPUE for weight was 6.4kg (sd=1.1, n=6) and the mean harvest size of 7.6 cm (sd=1.1, n=40) in December 2006. At the East site, 24 empty tests were found along the transect line with a mean diameter of 6.0 cm (sd=0.8, n=24) and a mean density was 0.03/m^2 (sd=0.05, n=3) in June 2007. The mean density for all sites was 0.06/m^2 (sd=0.05, n=3) compared to 0.005/m^2 (sd=0.004, n=10) in 2003.

Actinopyga sp.

The mean CPUE for numbers of *Actinopyga* sp. was 25 (sd=25, n=12) and the mean CPUE for weight was 2.8kg (sd=2.8, n=17). The mean size was 11.1cm (sd=3.5, n=124). This species was not found at the South site. The West and East sites significantly differed for mean CPUE for numbers and weight. At the West site, the mean CPUE for counts was 8.2 (sd=16.6, n=6) compared to 38.5 (sd=24.7, n=6) at the East site. The mean CPUE for weight was 0.53kg (sd =0.7, n=6) at the West site and 3.2kg (sd=3.0, n=14) at the East site. The mean size was 9.8cm (sd=2.3, n=28) at the West site and 11.5cm (sd =3.8, n=96) at the East site. The mean density was 0.003

(sd=.006, n=6) at the West site and 0.10 (sd=.006, n=6) at the East site. The mean density for both sites was 0.05/m^2 (sd = 0.11, n=12) and lower than the mean density of 0.07/m^2 in 2003.

Stichopus vastus

For all sites, the mean CPUE for numbers was 22.4 (sd=23.8, n=40) and the mean CPUE for weight was 3.2kg (sd=3.8, n=18). The mean CPUE for numbers, significantly differed (H=41) between sites as follows:West:30.2 (sd=25.7, n=29); South: 4.8 (sd=16.9, n=6); and East:11.9(sd=6.1, n=5). The mean CPUE for weight significantly differed (H=19) between sites as follows: West:3.8kg (sd=4.3,n=12); South:2.0kg (sd=3.0, n=3): East:1.2kg(sd=0.6, n=3). Densities significantly differed (H=31) between sites as follows: West: 0.05(sd=0.07, n=6); South: 0.92 (sd=0.86, n=6); and East: 0.009(sd=0.008, n=6). The more accessible areas of the West site had lower mean CPUE for numbers 1.0 (sd= 0.9, n=3) and weight 0.2kg (sd=0.09, n=3) than less accessible areas with a mean CPUE for counts of 19.9 (sd= 18, n=8) and a mean CPUE for weight of 7.0kg (sd=4.6, n=6). Higher mean densities of *S. vastus* were also found at the South site in the 2003 survey (Table 3). The mean size for all harvested *S. vastus* was 17.9cm (5.0, n=17) compared to 14.7cm (sd=2.3, n=1,388) in 2003.

Table 3 *Stichopus vastus* density (ct/m^2) with the standard error (SE) and sample number (n)

	2006-2008(ct/m^2)	2003 (ct/m^2)
West	0.01(0.01) n=2	0.05(0.07) n=6
South	0.94 (0.93) n=3	0.80(0 .76) n=3
East	0.01(0.003) n=2	0.02(0.003) n=2)
Mean	0.41(0.73) n=7	0.24(0.48) n=11

Holothuria scabra

For all sites, the mean CPUE for numbers of *H. scabra* was 6.7 (sd=5.8, n=10) and the mean CPUE for weight was 1.3kg (sd=1.2, n=10). The CPUE for numbers (H=16) and weight (H=15.7) significantly differed (the Kruskal-Wallis critical value$_{.05[2]}$= 5.25) between sites. The mean CPUE for numbers at each site was as follows: West: 10.7 (sd=4.7, n=5); South: 5.0 (sd=5.7, n=2); and East: 1.1 (sd=0.6, n=3). The mean CPUE for weight at each site was as follows: West: 2.1kg (sd=0.9, n=5); South: 1.2kg (sd=1.4, n=2); East: 0.2kg (sd=0.1, n=3). The mean density for all sites was 0.009/m^2 (sd=0.009, n=8) compared to 0.005/m^2 (sd=0.008, n=12) in 2003. The mean length was 14.5cm (sd=2.4, n=29) compared to 14.9cm (sd=13, n=6) in 2003 and 16.8cm (SE=0.6, n=17) for the 2007 SPC survey.

Lambis lambis

Lambis lambis had a mean size of 12.4cm (sd=2.3, n=13) and a mean weight of 0.3kg. The mean CPUE was 0.8 (sd=1.2, n=22). The CPUE for counts significantly differed (H=10, $X^2_{.05[2]}$ value=5.99), between sites as follows: South: 1.4 (sd=0.9, n=8); East: 1.2 (sd=0.7, n=4); and West: 0.6 (sd=0.3, n=4). The mean density of 0.004/m^2 (sd=0.003, n=3) was similar to the mean density of 0.005/m^2 (sd=0.004, n=10) in 2003. The mean size was similar to the 2007 PROCFish study by SPC.

Market Value

The mean purchase price for several invertebrate species between 2000 and 2007 was $6.00/kg at a local market. The mean annual sales were as follows: *Actinopyga* sp.: 1098kg, *T. crocea* and *T. maxima*: 148kg and *S. vastus*: 99kg. The retail values in 2008 were as follows: *T.crocea* and *T. maxima* meat: $10kg^{-1}, *S.vastus*: $10.80kg^{-1}; *H. scabra*: $10.50kg^{-1}; *T. gratilla*: $11kg^{-1}; sliced *Actinopyga* sp.: $6.50kg^{-1} and minced: $4kg^{-1}. The time required to harvest 1kg of these species based upon the mean CPUE for weight (not including transport and processing time) is as follows: *T.crocea* and *T. maxima* meat: 1.6h, *S.vastus*: 0.33h; *H. scabra*: 0.75h; *T.gratilla*: 0.18h and *Actinopyga* sp.: 0.3h. Revenues generated from hourly harvest ranged from $13 for *Actinopyga* sp. to $61 for *T. gratilla*. Commercial production showed an increasing trend for Yano's market (Evelyn Yano, unpublished data, 2008). Increased local demand for these delicacies by residents and visitors puts pressure on this fishery and some are illegally exported. Currently there are no national regulations for *S.vastus* or *T. gratilla.*

Discussion

The harvest rates of selected invertebrates showed high percent declines compared to a qualitative survey 17 years ago (Matthews and Oiterong 1991). The percent declines in harvest rates compared between 2007 and 1991 were as follows: embedded *Tridacna* species (60%), *H. hippopus* and other *Tridacna* species (75%), *S. vastus* (50%) and *H. scabra* (80%). The Belau Mariculture Demonstration Center (BMDC) produces clams that reach maturity within 5 years and have recommended minimum sizes to harvest wild clams. The size at first maturity of cultured clams and the recommended harvest sizes for wild clam populations in Palau are as follows: *T. crocea* (8cm; 12cm), *T. maxima* (10cm; 12cm), *H. hippopus* (12cm; 18cm), *T. squamosa* (18cm; 18cm) and *T. derasa* (30cm; 35cm) (Lawrence Sumer-BMDC supervisor, pers comm. 2008). Jameson (1976) observed *T. maxima* spawning at sizes ranging from 11cm to 13cm. The mean size of harvested *T.*

crocea and *T. maxima* in this study were lower than the recommended harvest size. The mean harvested size for *H. hippopus* was within BMDC's recommended size range and similar to the mean size in the 2007 SPC study. *Tridacna crocea* and *T. maxima* clams are good indicator species of reef health for *Porites* spp. dominated reefs because they are common and accessible, have a colorful mantle, and leave a depression after extraction, enabling easy detection and measurement. *Hippopus hippopus* is a potential early indicator species of overharvesting because women prefer it because it is easier to harvest and has larger meat than the embedded clams. However it is found in lower densities. Women transplanted smaller *H. hippopus* to depleted areas.

In the Philippines, densities of *T. gratilla* dropped from 0.11-2.14/m^2 to 0.02 to 0.07/m^2 prior to the collapse of the urchin fishery in 1992 (Juninio-Meñez et al. 1998). In this study, the mean density was 0.06/m^2 and 0.005/m^2 in 2003, which were similar to the threshold densities in the Philippines prior to its collapse. The mean diameter of harvested *T. gratilla* was similar to sexually mature cultured *T. gratilla* (6cm) in the Philippines and wild populations in other regions (Juinio-Meñez et al. 1998). In Palau, sea urchins were more abundant from November 2006 to March 2007 and were observed spawning in synchrony during April 2007. Breeding peaks were between September and November in the Philippines and Taiwan, (Chang-Po and Ku Hsiung 1981). Algal food availability (Conan and Sloan 1989) and macroalgae preferences (Stimson et al. 2007) were correlated to *T. gratilla* abundance and distribution. Temperature and photoperiod changes acted as cues to induce spawning in *T. gratilla* (Vaïtilingon et al. 2005). Coral (Penland et al. 2004), plants (Borchert 2005, Kitalong 2008), *Acanthaster plancii* (Idip 2003) and *Siganus fuscescens* (Kitalong et al. in prep) exhibited reproductive peaks that correlated with annual spring and fall maxima in solar insolation. Fisherfolk have observed that *T. gratilla* become buoyant and move in masses from reef to reef. They believe that urchins live in deep water and emerge to the shallows to breed. We found only empty tests in June at the East Site. During extensive surveys during May 2007 and the summer of 2003, only 4 and 3 urchins were found respectively (SPC 2007, Kitalong 2003), suggesting seasonality for this species in Palau.

Actinopyga sp. used coral as a refuge during daylight and emerged at night at the West site. *Actinopyga* sp. harvest rates were similar to estimated rates 17 years ago (Matthew and Oiterong, 1991). The mean size of harvested *Actinopyga* sp. in this study was 11cm which was smaller than the size of mature *Actinopyga echinites* (12cm) found by Conand (1982). In this study, *Actinopyga* sp. were common at the East site however women limited their harvest effort for *Actinopyga* sp. because it required several hours to process its edible endodermis. Women preferred to collect *H. hippopus*, *T. gratilla* and *H. scabra* which were easier to process.

The mean size of *S. vastus* decreased from west to east in this study and in 2003. Women said they preferred harvesting *S. vastus* at the West site because it "tasted better" than at the other sites. Their preference is reflected in the high CPUE rates yet lower densities at the West site compared to the South site. The author and fishers have observed a decline in populations at accessible sites. Women traditionally slice *S. vastus* into 2 or 3 sections and scatter them onto the depleted areas. *Stichopus vastus*(?) spawned during April 2003 at a southern reef.

The mean size of harvested *H. scabra* in this study compared to other countries indicates that they have not reached sexual maturity. *H. scabra* are known to emerge from the sediments on incoming tides at dusk. Juveniles burrow during sunrise and emerge during sunset to feed (Mercier et al. 1999). We observed similar behaviour in this study. The decline in *H. scabra* has been attributed to overharvesting and pollution from land based sediments and other pollutants. Golbuu et al. (2003) found high rates of sedimentation in Airai Bay.

Small patchy populations require measurement at fine spatial and temporal scales with input from resource users to better estimate and detect population change. Fishers are observing more restricted habitats and smaller sizes for most invertebrates. Mobile GPS tracking units locate specific habitats for targeted species for future monitoring. Experienced gleaners showed the less experienced gleaners the proper harvest sizes and how to harvest embedded clams without damaging corals. Sustainable harvesting skills must be taught in the field. Teamwork between managers and fishers enhanced implementation of this study and the exchange of valued information which provided more management options. These results can be used to educate the community; set size limits, threshold densities, seasonal harvest periods and site closures of important pre-spawning and spawning grounds. These key invertebrates can serve as potential indicators of reef health. Their functional roles as grazers, aerators of sediment and processors of organic matter are essential in maintaining a healthy reef.

During village meetings, the community recommended the following actions: stop dredging the inner reef areas, protect important areas for each

village, restrict boat traffic, reduce or stop sources of pollution, conduct more studies, develop sustainably, involve communities in activities and employ more conservation officers. Currently protected areas are being demarcated and Airai conservation officers are monitoring and enforcing marine laws with their local research, enforcement and community partners.

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Examination of Algal Diversity and Benthic Community Structure at Palmyra Atoll, U.S. Line Islands

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Abstract. Palmyra Atoll National Wildlife Refuge is the second largest atoll under U.S. jurisdiction in the Pacific Ocean. Until recently, little was known about benthic communities and, more specifically, algal abundance at Palmyra aside from species lists generated during 1916, 1955 and 1959 expeditions. Since 2000, NOAA's Pacific Islands Fisheries Science Center, Coral Reef Ecosystem Division has conducted six rapid ecological assessment surveys to monitor and gather baseline data for reefs surrounding Palmyra. Additionally, Scripps Institute of Oceanography documented benthic community structure on Palmyra's reefs during a 2005 expedition. This study reports on the current state of knowledge for benthic community composition and algal community structure across the atoll. Benthic communities in fore reef areas (10 m depth) were dominated by reef builders (coral and crustose coralline algae) covering ~50% of the benthos, while turf algae, macroalgae, soft corals, and other invertebrates covered the remaining surfaces. Species of *Halimeda*, *Lobophora*, *Dictyosphaeria*, *Galaxaura* and *Dichotomaria* were among the 5 most common macroalgae recorded. Overall, the reef communities around Palmyra appear relatively healthy. Developing comprehensive species lists and continuing benthic monitoring will allow reef managers to detect ecosystem changes, including possible introduced and invasive species.

Key words: algae, benthic community, coral, monitoring

Introduction

Palmyra Atoll, located in the northern portion of the Line Island Archipelago, is situated 1692 kilometers southwest of the Hawaiian Islands in the intertropical convergence zone (Maragos et al. 2008, Miller et al. 2008). It is the second largest of 10 atolls under US jurisdiction, and is composed of 182 and 6,277 hectares of emergent and submerged habitat, respectively (Handler et al. 2007; Rohmann et al. 2005). Although composed of several vegetated islets, Palmyra Atoll's geographically remote location has never fostered permanent human population. Thus, the atoll remained in near pristine condition until the early 1940s when it was turned into an US military airbase during World War II. An airstrip was constructed by dredging the central lagoon and using the debris to connect existing islets. A large ship channel was also dredged to allow military vessels to enter the calm lagoonal waters.

In 2000, Palmyra's largest island (Cooper Island) was purchased by the Nature Conservancy and a research station was built (Miller et al. 2008). In 2001, all remaining areas of Palmyra Atoll (excluding Cooper Island) were purchased by the US Fish and Wildlife Service and were designated a National Wildlife Refuge, thus increasing Palmyra's protected status (Miller et al. 2008). A recent study investigating the fish and benthic communities of the northern Line Islands found that the reefs of Palmyra and the more northerly Kingman Reef were characterized by high apex predator biomass in similar proportions to those found in the uninhabited Northwestern Hawaiian Islands (Sandin et al. 2008; Stevenson et al. 2007). Therefore, in spite of previous environmental changes made by the US military, 60 years devoid of human disturbance and Palmyra Atoll's distance from large human populations have allowed marine communities around the outside of the atoll to remain in a reasonably pristine state (Brainard et al. 2005; Sandin et al. 2008), offering a relatively unperturbed marine system for study.

Despite several early species lists (Rock 1916, Dawson et al. 1955, Dawson 1959), little has been reported about algal communities surrounding Palmyra Atoll. New species of marine algae from Palmyra were reported through the 1960s (Hollenberg

1968) and recent coral community monitoring has been performed (Williams et al. 2008; Maragos et al. 2008; Miller et al. 2008). However, no comprehensive analysis of algal community composition has yet occurred.

The goals of this study were to use two of the most comprehensive data sets available on benthic community composition of Palmyra's reefs to examine spatial patterns in functional group abundance, and more specifically, macroalgal abundance around the atoll. NOAA's Pacific Islands Fisheries Science Center (PIFSC), Coral Reef Ecosystem Division (CRED) has been examining benthic community biodiversity and abundance on reefs surrounding the atoll in 2000 and 2001 and biennially from 2002 to 2008. Additionally, in 2005, a comprehensive study of Palmyra reef communities was conducted by Scripps Institute of Oceanography (SIO; Sandin et al. 2008). Using these two complementary data sets, benthic communities were examined to (1) understand spatial patterns in percent cover of benthic functional groups, and (2) increase knowledge of macroalgal community composition. These results will be used as a baseline for future monitoring of Line Islands reefs, providing valuable insight for management efforts investigating the role played by algal communities at Palmyra Atoll (National Marine Fisheries Service, 2006).

Site Number	Location	Latitude	Longitude
PAL-01	S Fore Reef	5N 52.183	162W 4.137
PAL-02	W Terrace	5N 52.952	162W 7.878
PAL-04	W Terrace	5N 52.424	162W 6.990
PAL-05	N Fore Reef	5N 53.761	162W 8.247
PAL-06P	W Terrace	5N 52.294	162W 7.098
PAL-09	S Fore Reef	5N 52.046	162W 5.751
PAL-10	W Terrace	5N 51.961	162W 2.909
PAL-11	W Terrace	5N 53.027	162W 8.006
PAL-12	N Fore Reef	5N 53.834	162W 6.459
PAL-15P	E Terrace	5N 52.221	162W 2.697
PAL-16P	W Terrace	5N 52.2913	162W 6.7377
PAL-18	N Fore Reef	5N 53.765	162W 8.899
PAL-19	S Fore Reef	5N 51.9929	162W 6.5716
PAL-20	N Fore Reef	5N 53.7906	162W 7.1567
PAL-21	N Fore Reef	5N 53.7494	162W 5.0846
PAL-25	S Fore Reef	5N 51.8394	162W 1.8743
PAL-26	W Terrace	5N 51.831	162W 7.6128
PALB1	S Fore Reef	5N 51.986	162W 6.558
PALB2	S Fore Reef	5N 52.156	162W 4.539
PALB3	S Fore Reef	5N 51.925	162W 2.378
PALB4	N Fore Reef	5N 53.745	162W 4.888
PALB5	N Fore Reef	5N 53.810	162W 6.019
PALB6	N Fore Reef	5N 53.358	162W 2.799
PALB7	N Fore Reef	5N 53.870	162W 3.769
PALB8	N Fore Reef	5N 53.761	162W 7.172
PALB10	S Fore Reef	5N 51.976	162W 3.483
PALB12	N Fore Reef	5N 53.805	162W 7.707

Table 1: CRED and SIO (indicated with a B) benthic survey sites at Palmyra Atoll.

Materials and methods

Field Location

Palmyra Atoll (Figs 1, 2, Table 1) is one of 11 atolls and low islands situated in the Line Island chain, and sits atop a shallow bank <100 m below sea level. Large shallow (<30m) reef terraces extend from the eastern and western shores, with northern and southern shores exhibiting relatively narrow strips of fore reef area that rapidly drop to abysmal depths (National Marine Fisheries Service 2006; Maragos et al. 2008; Miller et al. 2008). Survey sites occurred between 3m to 15m depths in both reef terrace and fore reef habitats (Figs. 1, 2, Table 1). No survey sites were located within lagoonal habitats, although survey sites PAL-04 and PAL-16P were located adjacent to a dredged channel leading to the SW corner of the lagoon (Table 1, Figs. 1, 2).

Benthic Surveys

Quantitative Rapid Ecological Assessment (REA) surveys were conducted across the atoll by two research organizations: (1) NOAA's CRED established 20 sites between 2000 and 2008, and (2) SIO conducted an expedition in 2005 that surveyed 10 sites. CRED sites were stratified across reef terrace and fore reef habitats at ~15m depths (when possible) (Table 1) and subsequent surveys emphasized previously surveyed sites. SIO sites were all located in fore reef habitats at 10m depths and were located at 2km intervals around the atoll (Table 1). At each site, 2 transect lines of either 25m (CRED) or 50m (SIO) were deployed and 12 – 20 photographs were taken using a photoquadrat (CRED = 0.18m^2, SIO = 0.54m^2) equipped with a digital still camera (Preskitt et al. 2004) along each line for a total area surveyed of 2 – 10m^2 per site. Photographs serve as permanent documentation of each site and were analyzed for benthic percent cover using the Coral Point Count with Microsoft Excel extensions (CRED; CPCe; Kohler and Gill 2006) or PhotoGrid (SIO) software to assign one hundred stratified random points to each digital image.

Benthic percent cover data were divided into the functional groups of coral, crustose coralline red algae (CCA), turf, and macroalgae. Macroalgal data were further identified to the lowest taxonomic level possible. To examine whether community composition (functional group abundance) was similar across habitats within each sampling year, one-way analyses of similarity were performed (ANOSIM; number of permutations = 5,000) using Bray-Curtis Similarity Indices (based on fourth root transformed abundance data) performed in PRIMER-

E v.6. Data between sampling years were not combined in spatial analyses. SIMPER analyses were performed to determine which functional groups and/or algal species were contributing the most to differences or similarities between sites.

Results

Benthic Community Composition

Benthic communities surrounding Palmyra Atoll were relatively homogeneous in terms of functional group abundance (Table 2). ANOSIM results examining benthic community composition between fore reef and terrace habitats found no differences within each sampling year (Global R (p-value): 2004 = 0.071 (0.003), 2006 = 0.15 (0.018), 2008 = 0.131 (0.0002)). Similarly, no differences were observed among sites (regardless of habitat) in 2004, 2005 or 2006 (Global R (p-value): 2004 = 0.214 (0.0002), 2005 = 0.174 (0.0002), 2006 = 0.098 (0.0002)); although ANOSIM site comparisons revealed differences in 2008 (Global R (p-value) = 0.351 (0.0002)). From 2004 through 2008, percent cover of macroalgae ranged from 3 to 58% (Fig. 1) (average = 19%), reef builders (coral and crustose coralline red algae) ranged from 10% to 71% (average = 45%), and turf algal cover ranged from 7% to 70% (average = 30%).

In 2008, individual pairwise *r*-values from the ANOSIM test revealed that the difference observed in Global R values stemmed from differences in benthic community composition between western terrace sites (PAL-02, PAL-04, PAL-05,and PAL-06) and the rest of the atoll (Figs 1, 2). SIMPER revealed *Halimeda opuntia* to exhibit a percent cover of 32% at PAL-02, accounting for 17 – 31% of observed dissimilarities between this site and all others (PAL-02 pairwise *r*-value range = 0.022 – 0.824, median = 0.279). PAL-04, located on the western reef terrace near the boat channel, contained a dense community of the red macroalga *Dichotomaria marginata* that covered 37% of the substrate. The alga was not observed elsewhere around the atoll, therefore driving statistical differences between PAL-04 and other sites (pairwise *r*-value range = 0.01 – 0.853, median = 0.333). SIMPER analysis revealed that *D. marginata* accounted for 21 – 59% of the dissimilarity between PAL-04 and all other sites. Similarly, PAL-05, located on the NE fore reef, was different from 6 of the 8 sites surveyed in 2008 (*r*-value range = 0.204-0.853, median = 0.638). At PAL-05, turf algae covered 70% of the substrate and accounted for 34 – 39% of the dissimilarity found between this and other sites. A corallimorph that covered 69% of the substrate at PAL-06 (and was not seen elsewhere around the atoll) contributed to 38 – 43% of dissimilarities observed (PAL-06 *r*-value range = 0.553 – 0.664, median = 0.633).

Site	Year	Coral	Turf Algae	CCA	Macroalgae
PAL-01	2004	14.33	24.67	28.42	32.57
	2006	19.58	40.00	18.50	19.25
	2008	16.25	44.17	19.42	18.25
PAL-02	2008	13.17	12.08	13.75	54.91
PAL-04	2008	6.92	20.17	10.00	62.49
PAL-05	2008	11.00	69.50	7.67	4.33
PAL-06P	2008	8.67	16.92	1.25	4.34
PAL-09	2004	29.42	32.00	18.00	20.51
	2006	20.83	33.00	11.83	32.83
	2008	16.33	17.33	12.50	53.76
PAL-10	2004	31.83	30.50	14.67	21.32
	2006	14.08	37.50	11.25	31.85
	2008	30.58	28.58	10.08	29.50
PAL-11	2004	25.50	24.58	24.58	24.75
	2006	30.17	24.25	13.67	29.92
	2008	22.58	23.25	27.58	25.5
PAL-12	2006	27.00	40.83	13.67	14.50
	2008	32.00	34.58	13.50	14.5
PAL-15P	2004	58.08	30.08	8.92	2.17
PAL-16P	2004	4.17	37.33	17.50	40.92
PAL-18	2004	65.50	23.00	4.50	7.00
PAL-19	2004	22.50	32.42	21.58	20.75
	2006	40.58	22.08	14.92	20.59
PAL-20	2006	31.58	39.50	21.92	6.75
PAL-21	2006	20.83	35.17	16.58	24.16
PAL-25	2004	52.42	33.08	9.08	4.83
PAL-26	2004	36.42	37.25	17.83	5.92
	2006	54.33	26.42	9.33	4.58
PALB1	2005	15.85	21.00	30.35	29.1
PALB2	2005	13.96	34.00	21.70	25.3
PALB3	2005	47.30	13.00	29.10	10.25
PALB4	2005	24.15	25.20	24.05	24.45
PALB5	2005	28.40	28.80	19.80	19.35
PALB6	2005	23.25	42.00	14.45	15.15
PALB7	2005	31.62	30.76	17.29	17.57
PALB8	2005	18.60	24.85	41.95	14.21
PALB10	2005	17.6	6.70	47.60	22.7
PALB12	2005	42.75	19.25	28.60	9.8

Table 2: Average percent cover of benthic functional groups from survey sites from 2004-2008. Average percent cover of photoquads for each site during each year are presented. Standard errors not shown because of space limitations. CCA = Crustose Coralline Red Algae.

In 2004, percent cover of live coral at site PAL-16P was only 4% (compared to an average of 37% around the rest of the atoll), and this functional group contributed to 13 – 47% dissimilarity between PAL-16P and other sites during this sampling year (PAL-16P *r*-value range = 0.257 – 0.734, median = 0.42). PAL-16P site has not been resurveyed since.

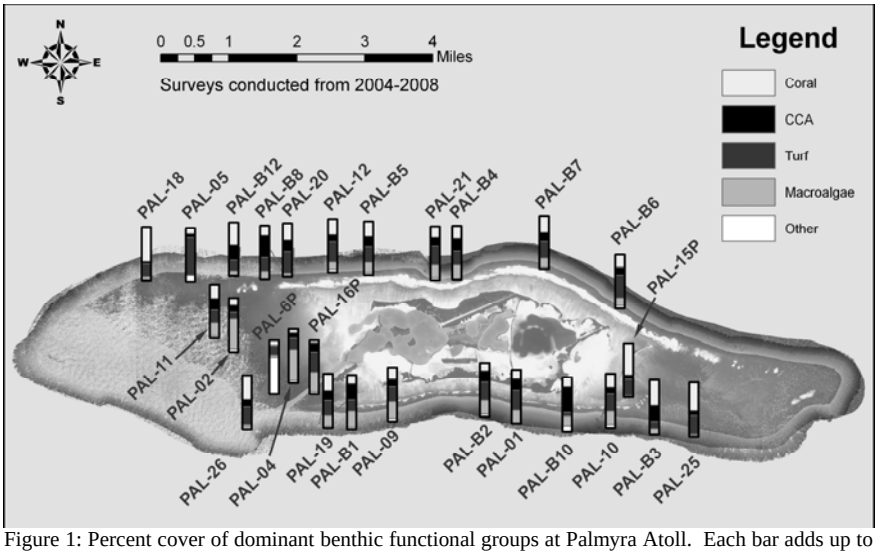

Figure 1: Percent cover of dominant benthic functional groups at Palmyra Atoll. Each bar adds up to 100% cover. Percent cover data from sites visited in multiple years were averaged (Table 2).

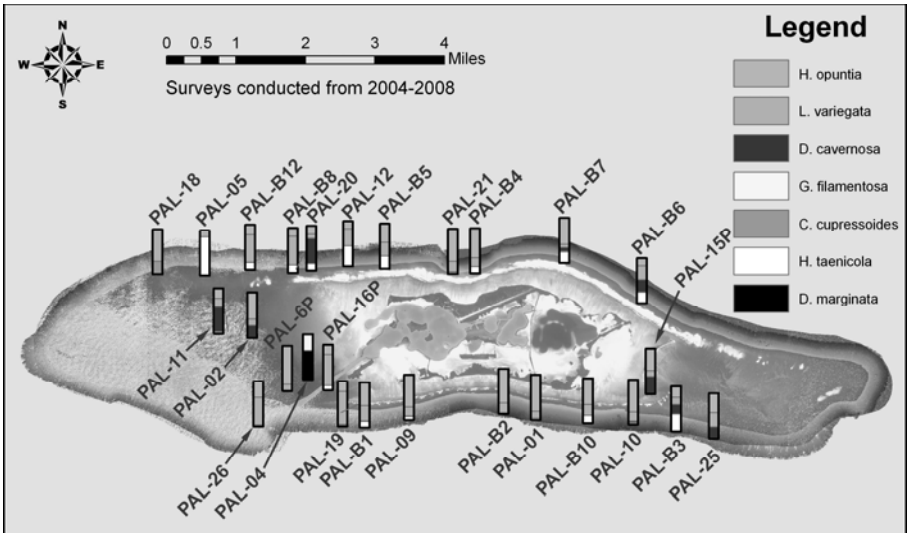

Figure 2: Relative abundance of select macroalgal species at Palmyra Atoll. Each bar shows percent contribution of each algal species to total macroalgal cover present at each site. Percent cover data from sites visited in multiple years were averaged (Table 2).

Algal Community Composition

Nineteen species of macroalgae were documented in photoquadrats at Palmyra Atoll. Of these, *Halimeda opuntia* was the most common, exhibiting a percent cover range of 0 – 46% (average = 8.2%). *Lobophora variegata* (percent cover = 0 - 27.7%, average = 5.3%), *Galaxaura filamentosa* (percent cover = 0 – 37%, average = 1) and *Dictyosphaeria cavernosa* (percent cover = 0 – 16.5%, average = 1.6%) were the next most common species.

ANOSIM analyses performed on macroalgal percent cover data (omitting all other functional groups) found algal communities to be relatively similar across the atoll (Global R: 2004 = 0.196; 2005 = 0.073; 2006 = 0.48; 2008 = 0.184, significance = 0.02%); however, PAL-04 (Fig. 2) showed a statistically significant difference in macroalgal percent cover from 5 of the 8 other sites surveyed in 2008 (*r*-value range = 0.623 – 0.997, significance.

Discussion

Overall, the outer fore reef areas of Palmyra Atoll remain healthy, presumably because of the lack of current anthropogenic influences. Benthic community structure is dominated by reef building organisms, primarily coral and CCA at 82% of sites. At only four sites did macroalgal cover exceed that of either reef builders or turf algae, a finding similar to that of a previous study at eight other relatively pristine Pacific reefs, where macroalgal cover was lower than that of coral at 67% of sites (Vroom et al. 2006). Findings at

Palmyra are also in line with those from Kingman Atoll, the closest island to Palmyra, where benthic cover was dominated by reef-building organisms in contrast to macroalgal dominated substrates around the more densely populated islands of Tabuaeran and Kiritimati (Sandin et al. 2008).

The high percent cover of the red alga *Dichotomaria marginata* and a corallomorph documented at PAL-04 and PAL-06, respectively, may be due to these site's close proximity (477m) to a 37m, steel-hulled, long line vessel which ran aground in 1991. Changes in algal communities have also been observed to occur in close proximity to a 1993 shipwreck on Rose Atoll (Schroeder et al. 2008). Rose Atoll was monitored by CRED from 2002 to 2006 and exhibits percent cover of opportunistic algal species two times higher at the shipwreck site as compared to sites further away. Additionally, Work et al. (2008) found high densities of the corallimorph, *Rhodactis howesii*, to occur within 1 km^2 of the Palmyra wreck suggesting possible correlations with nutrient availability and changes in benthic communities. Further research examining nutrient levels at PAL-04 and PAL-06 are needed to definitively link the shipwreck at Palmyra Atoll with increased percent cover of *D. marginata* and corallomorphs.

Finally, the protected status of Palmyra Atoll guarantees that the surrounding reef communities will be spared from any direct human impacts, allowing Palmyra Atoll and other remote reefs to serve as important natural laboratories for understanding the impacts of climate change on coral reefs.

Acknowledgements

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Assessment of Resilience in *Montastrea faveolata* Inside and Outside of a Marine Park

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Abstract

Lesion regeneration (wound healing) was used as a proxy for assessing organismal resilience in the coral, *Montastrea faveolata*. Potential effects of wounding on quantum yield determined by PAM fluorometry were also examined. In a mesocosm experiment, surface lesions were imaged daily over 15 days to develop healing curves. Quantum yields were obtained daily using a PAM fluorometer (2 mm fiberoptic). Results demonstrated that 50% healing occurred in 5-6 days. Lesion healing was compared between an unprotected location near Lee Stocking Island (Exuma, Bahamas) and in the Exuma Cays Land and Sea Park using coral cores deployed to field arrays at each site. Lesions, images and PAM measurements were made on day 0 and again 5-7 days later. These procedures were repeated in the winter, spring and summer of 2007 and winter, 2008. There were no seasonal differences in healing rates or between genotypes. No consistent effects of site, time of year or wounding were seen on quantum yields of surrounding tissue, however, newly formed tissues had significantly lower yields than measured in undamaged tissues. Healing was significantly greater at the Park site in the winter of 2007 but there were no site differences in spring or summer.

Key words: corals, organismal resilience, coral stress, wound healing

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Introduction

There is a broad consensus that reef corals are threatened by a variety of anthropogenic and natural stressors. This has increased interest in assessing organismal stress and resilience. Some approaches, such as measuring coral growth, are inexpensive and simple but usually take considerable time for results to be obtained. Other methods are more rapid and can also provide very specific information. These include measurement of photosynthetic performance and/or competence, determination of calcification rates and a broadening array of cellular biomarkers. Drawbacks include higer costs, technical expertise requirements or access to a suitable laboratory.

Several investigators have examined lesion regeneration (wound healing) in response to environmental conditions such as turbidity and sedimentation (Lester and Bak 1985; Meesters et al. 1992; Cróquer et al. 2002), bleaching (Meesters and Bak 1993; Mascarelli and Bunkley-Williams 1999; Fine et al. 2002) or pollution (Williams 1994). Results from these and other studies (Bak et al. 1977; Meesters et al. 1994; 1997; Nagelkerken et al. 1999; Oren et al. 2001) suggest that wound healing reflects overall physiological condition and response to the environment. These findings led to proposals (Meesters and Bak 1994; Williams 1994) and projects (Fisher et al, 2007) to use lesion regeneration as a measure of coral condition.

In addition to environmental conditions and species (Bak and Van Es 1980; Nagelkerken and Bak 1998; van Woesik 1998; Nagelkerken et al. 1999), results from previous studies have demonstrated that lesion size and shape have a profound effect on healing rates (Meesters et al. 1994, 1997; Oren et al, 1997; Loya 1997; van Woesik 1998; Lirman 2000; Hall 2001). Large lesions heal more slowly, are more susceptible to enlargement by grazers (Cróquer et al. 2002) or colonization by epibionts (van Woesik 1998; Hall 2001), in some cases preventing complete closure (Mascarelli and Bunkley-Williams 1999; Cróquer et al. 2002) or allowing establishment of disease such as black-band (Cróquer et al. 2002).

To be useful for comparing coral resilience over time and space, the lesion regeneration method should minimize impact on corals and be standardized with respect to species, colony size and environment, lesion characteristics and measurement. Here we present and propose a Coral Resilience Assay that meets these requirements and is rapid, inexpensive and requires little technical expertise. The method is applied to examine whether corals in the Exuma Cays Land and Sea Park, a well-established marine protected area, have greater resilience than in unprotected areas.

Methods

Mesocosm Experiment

Four 1" diameter cores were extracted from each of four *Montastrea faveolata* colonies using a carbide core bit. Colonies had been maintained in a 16,000-liter tank 23-28 months, thus all had been exposed to similar environmental conditions. The mesocosm experiment described here was also conducted within this tank. Cores were mounted to 2" square plastic PVC or ABS plates using epoxy (Quick Fix 2300; Progressive Epoxy Polymers, Inc.) and allowed to recover for several days.

Digital plan images of each core were recorded (Nikon 3100; 2048x1536 pixels) with a 1-cm scale and pulsed-amplitude modulated fluorometry (Walz Diving PAM) measurements performed. These tasks were accomplished using the COral Resilience Assessment System (CORAS; see Mueller 2008 for details and images). The CORAS is a submersible platform that allows repeatable positioning of the cores in three axes (X-Y-Z). Using a 2-mm optic fiber, two PAM measurements were made at each of the four cardinal points (N, E, S & W). The first was 3 mm from the area to be wounded (see below) and the second 5 mm distal to the first. Angle of the fiber optic was 45 and it was held 3 mm above the coral surface. Procedures were conducted at mid-day and corals were not dark adapted, thus, yield measurements are apparent.

On 9/24/06, a 5/16" carbide end mill was used to make a ~0.5 cm^2 wound in the center of three cores from each colony to a depth of 2-3 mm; the fourth core was left as a control. All cores were re-imaged and PAM measurements made immediately following wounding. All were imaged daily until 10/10/06, 16 days after wounding. Wound areas in each image were calculated using SigmaScan Pro (V.5).The PAM measurements described above were conducted daily 9/24-28/06 then on 9/30/06 and 10/2/06. On 10/9/06, PAM measurements were made in just two locations on each coral, on a portion of healed lesion and on tissue that had not been injured. Temperature and conductivity (salinity) were recorded at 10 min. intervals (Alec Electronics).

Site Comparison with Cultured Corals

Twelve 1" cores were removed from six cultured *M. faveolata* colonies (different from those in the previous experiment), mounted on PVC plates and secured to six PVC field arrays (Fig. 1). The six colonies were each represented by two cores on each array; the exact cores and their positions within each array were randomly selected. Three arrays were installed Jan., 2007 at Jeep Reef (EX01) in the Exuma Cays Land and Sea Park (ECLSP; a no-take area) and three at North Norman's Reef (EX02) near Lee Stocking Island (unprotected). Both sites were on sand near patch reefs with similar depths (6-7 m). One array at each site was instrumented with a conductivity-temperature logger and a depth recorder (Alec Electronics).

Over the following year, coral responses to wounding were seasonally examined (winter 2007, spring 2007, summer 2007 and winter 2008). Coral cores were retrieved from the arrays and brought to a shore-side seawater system overnight. They were imaged and PAM measurements conducted. After wounding, these procedures were repeated and the cores returned to the field arrays for 5-7 days. They were again retrieved for imaging and PAM measurements. Procedures were similar to that described above with the following differences: images were made with a higher resolution camera (Sea & Sea DX8000; 3264x2448 pixels), four (rather than 8) PAM measurements were made per core at each of the cardinal points and wounds were made with a 5/16" stainless steel rod. Instruments were serviced and downloaded during each assessment period.

Site Comparison with In Situ Corals

Wound healing assessments were conducted in February and March/April 2008 near Warderick Wells (WW) in the ECLSP and in Elizabeth Harbour (EH) near George Town, the largest population center in the Exumas (Feb. April, and May, 2008). Within both study areas, four mature *M. faveolata* colonies of similar size and condition were haphazardly selected at each of four similar patch reefs (16 colonies within each study area). Four wounds per colony (at least 10 cm apart) were made on the colony top surface with a 5/16" punch. Wounds were imaged (Canon SD800; 3072x2304 pixels) with a 1-cm scale and repeated 5 days later. Wound areas were assessed using Coral Point Count with Excel Extensions V 3.5 (Kohler and Gill 2006).

Results

Mesocosm Experiment

The end mill wounds were somewhat ragged, thus, the day after the wounding (9/25/06), when tissue had sloughed off, was used as the basis for initial lesion size. Thus, the number of days shown on the graphs is 15 rather 16 days. Fig. 1 shows an example of the healing observed the experiment. The first wounds healed 100% by day 13 and by the end of the experiment (day 15), five of the 12 wounds had completely healed. Three months later (data not shown), all but two cores had completely healed.

The healing of the three replicate cores was averaged to represent each colony and the four colonies averaged to produce grand means for each day. The recovery curve for all cores over the initial 15 days is shown in Fig. 2. The 50% lesion recovery time was just over 5 days.

No effects of wounding were observed on the apparent quantum yields of tissues proximal (~3 mm) or distal (~8 mm) to the lesion. However, newly healed tissues had significantly lower apparent quantum yields than the adjacent undamaged tissues.

Site Comparison with Cultured Corals

Over the 1-year of this experiment, tissue loss resulted in morbidity that precluded making all four PAM measurements or complete mortality of the coral core. Based on the initial 72 cores, the morbidity rate over the year was 35% and the mortality 14%. Corals fared most poorly during the period (8/07-1/08) prior to the last assessment. Factors responsible could include maximal temperatures of 31-32º C at both sites (8/07) and Tropical Storm Noel (11/07) that produced salinities as low as 30 ppt (EX02; 34 ppt at EX01).

Normalized healing (% of initial lesion day^{-1}) at all sites in all seasons are compared in Fig. 3. Because no field measurements were made in the Fall, the mesocosm experiment results ("LSI") are shown for comparison. With the exception of healing at EX02 in the Winter of 2007, averaged healing at all locations throughout the year was typically 9-11 % day^{-1}. The only significant site difference was in Winter, 2007 where wounds at EX01 healed faster than at EX02.

Overall, there were no seasonal effects. ANOVA results indicate that there were no genotypic differences. Apparent quantum yield (F_v/F_m) values typically ranged from 0.4-0.6 under subdued mid-day lighting. No significant differences were attributable to sites, season or genotype.

Site Comparison with In Situ Corals

Regeneration of lesions on *M. faveolata* colonies in situ were generally similar to those seen on the 1" cores in the previous experiments. During the February 2008 assessment, coral lesions at the Warderick Wells sites healed significantly faster than those in Elizabeth Harbour (Fig. 4). The mean healing rate for all colonies (n=16) at the four WW sites was 11.74±3.09 % day^{-1} and for the EH sites 7.63±3.72 % day^{-1}. A repeat of the procedures in March/April on the same colonies did not show a significant difference between the study areas (WW: 8.51±3.78 % day^{-1}; EH: 7.28±3.30 % day^{-1}). The regeneration rates at the WW sites were lower during the second assessment but not significantly so.

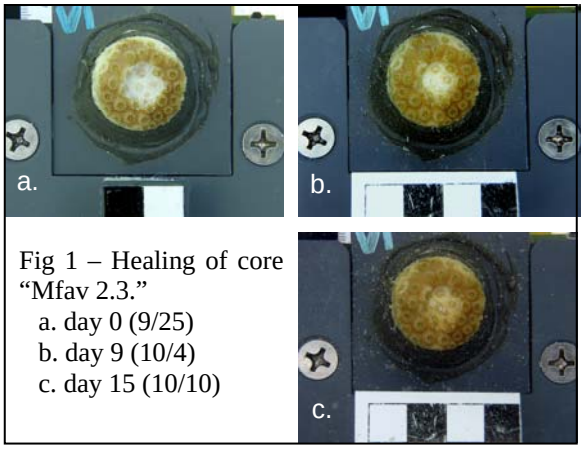

Fig 1 – Healing of core "Mfav 2.3."
a. day 0 (9/25)
b. day 9 (10/4)
c. day 15 (10/10)

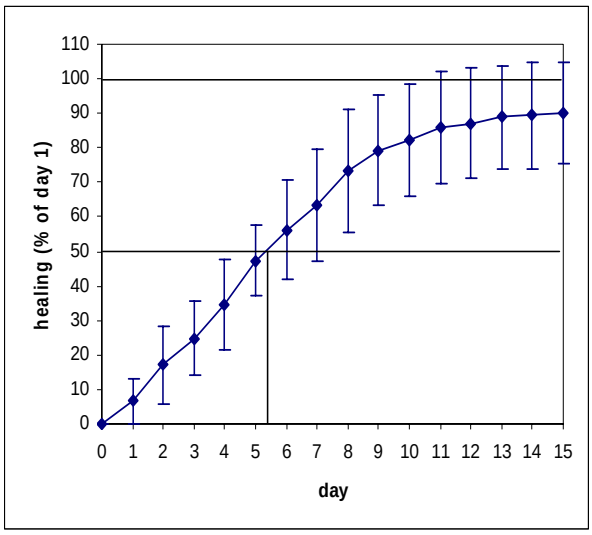

Fig. 2 – % healing of all corals (mean ± SD; n=4).

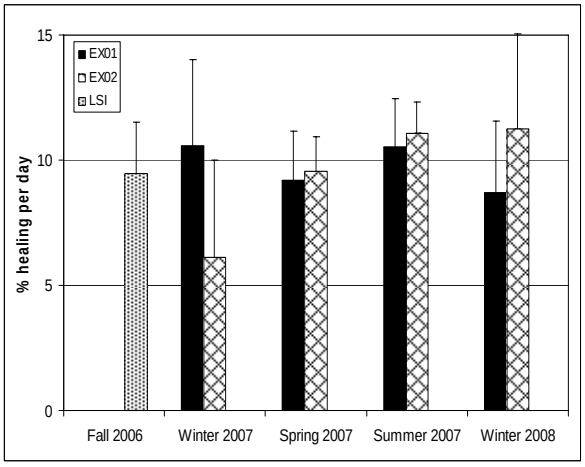

Fig. 3 – Wound healing at field sites (n=6; EX01 & EX02) and in the mesocosm experiment (n=4; Fall, 2006). Mean ± SD.

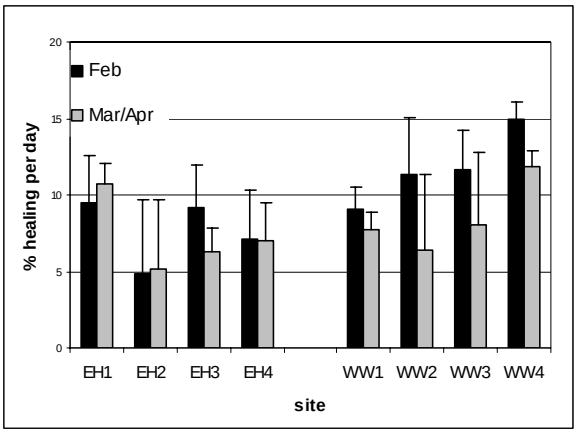

Fig. 4 – % healing of *in situ* colonies at Elizabeth Harbour (EH) and Warderick Wells (WW) sites in Feb. and Mar./April, 2008. Bars represents the mean ± SD of 4 colonies (each with 4 lesions) at each patch reef site.

Discussion

The Coral Resilience Assay has been applied to corals in several experimental settings: cores derived from cultured parents in mesocosm- and field-based experiments and colonies *in situ* on patch reefs. The Assay resulted in consistent tissue regeneration times. Results showed generally faster tissue regeneration than in other studies with corals within the *Montastrea annularis* complex, some of which founds tissue regeneration times requiring months (Mascarelli and Bunkley-Williams 1999; Cróquer et al. 2002; Fisher et al. 2007).

Using a small (0.5 cm^2) lesion maximizes the possibility that the lesion will completely heal, thus preventing establishment of epibionts or disease, and allows for results to be obtained quickly. A five-day regeneration period was found to be suitable for producing enough tissue to provide a good signal-to-noise ratio but not so long as to allow complete lesion healing that would preclude quantitative assessment

M. faveolata was selected as a standardized species because of its importance to Western Atlantic reefs and its broad distribution across the province. In addition to broad distribution, *M. faveolata* is commonly found on all reef types encountered in the area and across a wide depth range. The surface of *M. faveolata* was found conducive to producing uniform lesions.

Field experiments with cores and *in situ* colonies did not find consistent differences inside or outside of the ECLSP. Two of the six assessments described (1 of 4 with cores; 1 of 2 with *in situ* colonies), did find significantly faster healing rates inside the ECLSP. Although both of these cases were in the winter months, no seasonal trends were apparent as other assessments in winter found no differences. Examination of apparent quantum yields did not reveal any effects of the lesions on adjacent tissues or seasonal effects. However, newly-formed tissues over the lesion did have significantly lower apparent quantum yields than adjacent, undamaged tissues.

To obtain useful results, the Coral Resilience Assay must be applied with special attention paid to making lesions of consistent type, size and depth (Oren et al. 1997; Hall 2001). In designing a field project, data from others strongly indicate that lesion position on the colony is important (Cróquer et al. 2002) and that colonies should be of similar size (Meesters et al. 1997) and depth (Nagelkerken et al 1999;Fisher et al. 2007).

The Coral Resilience Assay is relatively inexpensive and all necessary equipment, excluding a computer for image analysis, can be obtained for less than $500.00. Suitable image analysis software (CPCe) is available as a free download from the National Coral Reef Institute, Nova University, Dania, FL (www.nova.edu/ocean/cpce/). Scientists and resource managers are encouraged to employ this standardized lesion protocol across the Western Atlantic Province to examine coral resilience and potential stress within and between regions. A booklet with detailed descriptions of the methods and data analysis methods is available from Conservation International (www.conservation.org).

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Decline of crinoids on the reefs of Curaçao and Bonaire, Netherlands Antilles

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Abstract. Since 1996 at the latest, comatulid crinoids on the leeward reefs of Curaçao and Bonaire have declined drastically in population size and diversity. Prior to this decline, five species inhabited the forereef slope from depths of 6 to >30 m. *Davidaster rubiginosa* and an undescribed species of *Davidaster* were common from about 6 to 15 m depth, and *D. discoidea* was common below about 15 m. *Nemaster grandis* and *Ctenantedon kinziei* were found in lower abundance at about 30 m depth. Transect data from Bonaire showed that a drastic decline in numbers of the *Davidaster* taxa occurred between 1989 and 1996. Transects in Curaçao in the late 1990s through 2001 documented a similar decline. In 2007, sites in Curaçao where species of *Davidaster* were formerly common were practically devoid of crinoids. In particular, *D. discoidea*, once the most numerous crinoid in these islands, has all but disappeared. The cause of this decline on both islands is unknown. It is possible that heating associated with the severe coral bleaching event of 1995 also affected the crinoids. As far as we are aware, reef crinoid populations across the broader western Atlantic region have not shown a similar decline.

Key words: Crinoids, Curaçao, Bonaire

Introduction

Underwater censuses conducted in the late 1960s showed that five species of comatulid crinoids (feather stars) were common on the leeward fringing reefs of Curaçao and Bonaire in the Netherlands Antilles (Meyer 1973). *Davidaster rubiginosa* (Pourtalès) and an undescribed species of *Davidaster* were common along the edge of the forereef slope from about 6 to 15 m depth, and *D. discoidea* (P. H. Carpenter) was common below about 15 m. *Nemaster grandis* Clark and *Ctenantedon kinziei* Meyer occurred in lower abundance at about 30 m depth. Subsequent work in Curaçao by Liddell in the late 1970s (Liddell 1980, Liddell and Ohlhorst 1982) found similar patterns in crinoid distribution and abundance. Quantitative censuses conducted in Bonaire by Llewellyn in 1989 (Llewellyn 1991, Llewellyn and Meyer 1991) found evidence of continuing crinoid abundance. Following a hiatus in observations, in 1996 Meyer was alarmed to find an apparent decline in the numbers of crinoids in both Curaçao and Bonaire. In 1999, Lask and Meyer resurveyed the Karpata reef in Bonaire where Llewellyn had inventoried every crinoid within an area of approximately 1000 m^2 in 1989 (Lask and Meyer 2001). Llewellyn reported a total of 70 crinoids within this sampling area, but the 1999 survey found a single crinoid. Lask and Meyer (2001) revisited 13 sites in Bonaire and compared crinoid occurrence with records from these and other sites accumulated over the period 1971-1996. The comparison showed that *D. rubiginosa* had become much less common and that *D. discoidea* had totally disappeared from sites where it had previously been common (see Fig. 1). In this paper we present a similar comparison of crinoid occurrence at sites along the leeward reefs of Curaçao in the late 1960s with surveys made since 2000, and changes in population density at one site, Jan Thielbaai, that Meyer surveyed in the late 1960s.

Materials and Methods

During the period 2000 – 2007, scuba dives at 14 sites along the leeward coast of Curaçao recorded crinoid occurrence to depths of about 30 m (Fig. 2). Nine of these 14 sites had been inventoried for crinoids during the late 1960s. These dives commenced just above the top of the forereef slope at about 6 m, descended to about 30 m, followed by a short traverse at the maximum depth, and return to the shallows along an oblique course across the forereef slope. Crinoids encountered were recorded by species and depth. The total reef area surveyed at different sites was variable.

A census within an area marked off into depth zones was conducted at the Jan Thielbaai site in 1968 – 69 (Table 1, Meyer 1973). This census extended from a depth of 8 m along the top of the forereef slope to 38 m and covered a total area of 1905 m^2. Crinoids were

recorded within marked depth intervals and the results were plotted as bathymetric changes in population density. In 2001 and 2007 a large areal census at this site was not repeated, but several strip transects were inventoried at different depths parallel to the reef slope at the same location of the earlier survey. In 2001, two transects each 50 m long were surveyed at two depths 10 m and 15 m, with two divers each surveying a band one meter above and below the tape, for a total of 200 m^2 per transect. In 2007, the census followed the AGRRA protocol of six transects, each 1 m wide by 10 m long, at a depth of 8 m along the top of the reef slope (Ginsburg 2005). Crinoids were also inventoried at Jan Thielbaai during 2000 – 2007 on several dives to about 30 m that did not control for survey area.

Results

In 1968-69, the most common crinoids, *Davidaster rubiginosa* and *D. discoidea*, could be found at most sites along the leeward reefs of Curaçao (Fig. 2) as well as Bonaire in 1971 (Fig. 1). At that time the undescribed species of *Davidaster* was considered to be an intraspecific variant of *D. rubiginosa*. Therefore the records of occurrence for *D. rubiginosa* for 1968-69 include forms now regarded to be a separate species. Only at the Jan Thielbaai site do photographic records and the tally of distinct variants of *Davidaster* (Table 1) provide a definite record of the undescribed species in 1968-69. Surveys conducted from 2000-2007 revealed that both *D. rubiginosa* and *D. discoidea* have declined in occurrence in Curaçao as they have in Bonaire (Fig. 2). One or both species have disappeared from several sites where they occurred in 1968-69.

The presence/absence data in Figure 2 alone do not convey the drastic decline in crinoid populations on the Curaçao reefs. Beginning in 1996, it was apparent that crinoid abundance was conspicuously reduced in Curaçao as it was in Bonaire (Lask and Meyer 2001). In 2001, we resurveyed the Jan Thielbaai site where Meyer had conducted a census of population density in 1968-69 (Table 1). Transects along the reef slope at 10 and 15 m covered depth intervals where crinoids had maximum density in 1968-69. *D. discoidea* was absent from these sampling areas, and both *D. rubiginosa* and *Davidaster* n. sp. were greatly reduced in numbers. *Nemaster grandis* was encountered in low numbers at 10 and 15 m, where it had been rare historically. This species was observed in greater numbers around 30 m depth. Although we were unable to obtain population density data at depths below 15 m, the *N. grandis* population appeared to be at levels similar to 1968-69. We revisited the site in 2002 2003 2004, and 2005, and found the same three species to be present. In 2007 we conducted an

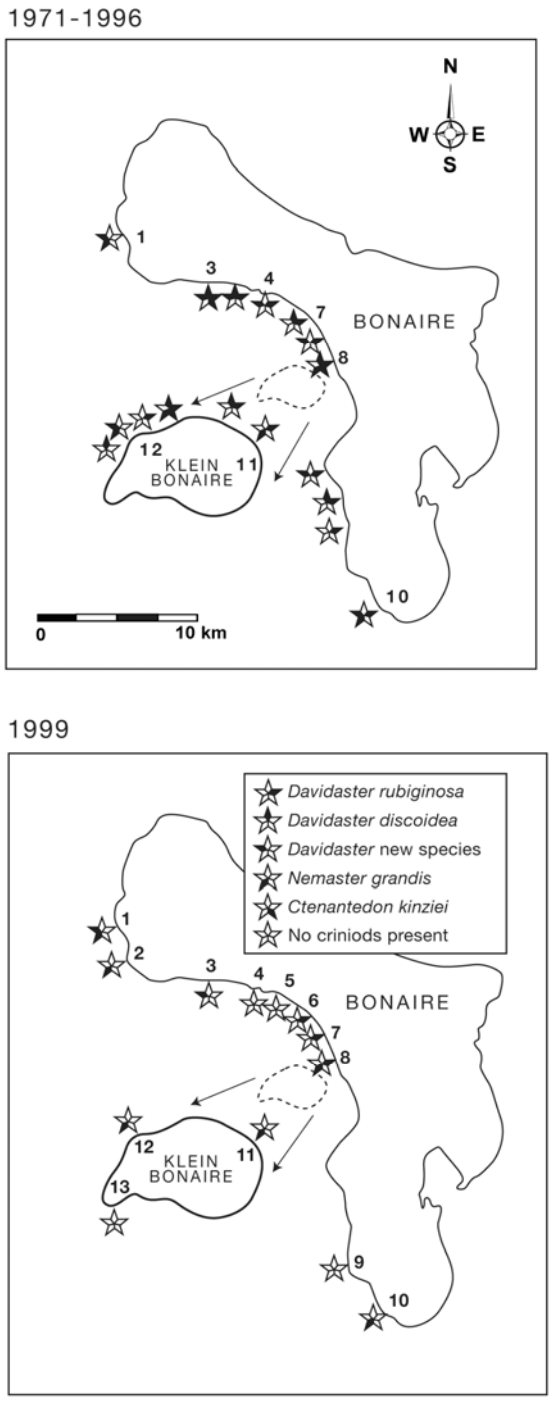

Figure 1: Crinoid species distribution at sites in Bonaire surveyed in 1971-96 (left), and in 1999 (above). Species occurrence is indicated by a black infilling of each of the five segments of the arrow symbol as shown in the legend. Modified after Lask and Meyer (2001).

AGRRA survey at Jan Thielbaai which included a series of transects at 8 m depth along the top of the reef slope. Although crinoids had not been very

common at 8 m historically, we were surprised not to encounter any crinoids at all in those sampling areas. In 2007 we also conducted AGRRA transects at Slangenbaai, another site where crinoids were very common in 1968-69. No crinoids were encountered within 6 transects, each 1 x 10 m, parallel to the reefslope at 8-10 m depth. Two individuals of *Davidaster* n. sp. were encountered outside these sampling areas at about 10 m depth. Clearly the population density of crinoids at this site was also drastically reduced from levels observed in 1968-69.

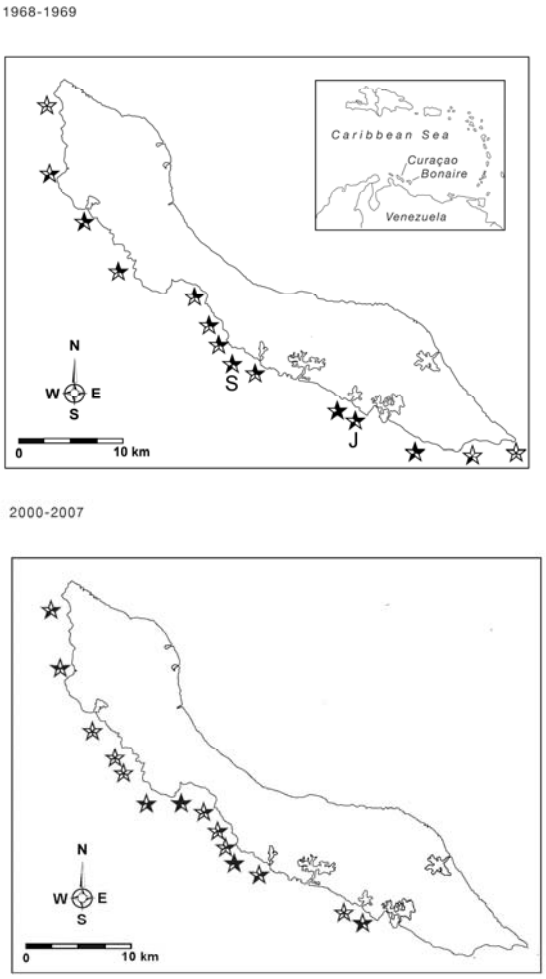

Figure 2: Crinoid species distribution at sites in Curaçao surveyed in 1968-69 (bottom of left column), and in 2000-2007 (above). Species occurrence as in Fig. 1. J = Jan Thielbaai, S = Slangenbaai.

Discussion

An alternative hypothesis is that the observed decline since the early to mid-1990s represents part of a long-term cyclical fluctuation in crinoid populations, occurring on a timescale greater than the 40 yr span of underwater observations of crinoids in the Netherlands Antilles. Although such a possibility cannot be discounted, we feel it is unlikely given the apparent stability in crinoid populations from the late 1960s until the mid-1990s. The possibility that the timing of our surveys could have coincided with some short-term seasonal fluctuation in crinoid populations is also unlikely because surveys prior to 1996 when crinoids were more numerous usually occurred during the summer, and surveys after the mid-1990s drop in crinoids were also taken in the summer months.

The exact timing and cause of the decline in crinoids on the leeward reefs of Curaçao and Bonaire remain unresolved. We know that the decline occurred between 1989 and 1996, but we do not know where or when the decline started or how rapidly it progressed, as there was a six-year hiatus in our visits to the islands. By 1996 it appears the decline was well underway as we did not observe any recently dead

depth (m)	*D. rub.*	*D. disc.*	*D.n.sp.*	*N. grd.*	date
8.5	0.009	0	0	0	1968
10.7	0.041	0.002	0.005	0.002	1968
13.7	0.038	0.019	0.005	0.005	1968
16.8	0.121	0.114	0.017	0.003	1968
21.3	0.01	0.13	0.01	0.005	1968
25.9	0.022	0.211	0	0	1968
29	0.025	0.2	0	0.013	1968
34.3	0.013	0.157	0	0.026	1968
10	0.005	0	0.003	0.003	2001
15	0.005	0	0	0	2001
8	0	0	0	0	2007

Table 1: Population density (individuals/m^2) at depth intervals along forereef slope at Jan Thielbaai, Curaçao. Depths for 1968-69 data are mid-points of depth intervals. *D. rub*. = *Davidaster rubiginosa*, *D. disc*. = *D. discoidea*, *D.* n. sp. = undescribed sp. of *Davidaster*, and *N. grd*. = *Nemaster grandis*.

crinoids in 1996. Because crinoids decay and disarticulate within a few days after death, it is likely that skeletal remains of crinoids would be rapidly dispersed in the reef sediments (Meyer and Meyer 1986). Unless an observer encountered moribund crinoids directly, it is quite possible that a mass die-off could pass unnoticed. In 2000, Lask observed a single moribund individual of *D. rubiginosa* at 11 m depth at one site in Curaçao, but was unable to obtain further information about the cause of this condition. The crinoid die-off may have followed a pattern similar to that of the mass mortality of the reef-dwelling echinoid *Diadema antillarum* (Bak et al. 1984). Meyer observed scores of moribund and recently dead echinoids when the mass mortality occurred in Bonaire in November 1983. By August of 1984 the skeletal remains of enormous numbers of echinoids had been effectively dispersed into the reef sediments (Greenstein 1989).

It is noteworthy that the crinoid decline occurred in both Curaçao and Bonaire. Curaçao has a human population about an order of magnitude higher than that of Bonaire, accompanied by a much higher degree of industrialization and coastal development. In contrast, Bonaire has little industrialization but increasing levels of coastal development. The entire fringing reef of Bonaire, contained within the Bonaire Marine Park, is one of the world's best-protected reef tracts. Because Curaçao usually lies downstream from Bonaire in the path of the Caribbean Current, pollution outfall from more-developed Curaçao will most likely be carried westward. Occasional reversal of the normal westerly flow could carry pollutants from Curaçao to Bonaire. It should also be noted that despite the increased likelihood of anthropogenic impacts on the Curaçao reefs, two sites where crinoids were surveyed, Jan Thielbaai and Slangenbaai, were both treated as control sites with little or no upstream coastal development in Nagelkerken's 2006 study of human impact and bleaching-related coral mortality.

One possible environmental perturbation that could have affected the crinoid populations on both islands was a protracted interval of seawater temperature elevation in late 1995. This thermal stress resulted in widespread coral bleaching and subsequent mortality along the leeward reefs of Curaçao and Bonaire (CARICOMP 1997; Nagelkerken 2006). In 1996, Meyer observed the ongoing effects of this bleaching as partial mortality and incipient recovery of coral colonies. Seawater surface temperature data reported by Nagelkerken for several sites along the leeward coast of Curaçao, including Jan Thielbaai, showed that temperatures rose in August and attained a maximum close to 30 deg. C by late September, returning to normal levels by late December. Although the physiological tolerance of comatulid crinoids for elevated temperatures is not well known, it is quite likely that many crinoids could have succumbed to this thermal stress. The fact that crinoids previously common along the shallower parts of the forereef slope, from 6- 15 m, seem to have been most severely reduced in numbers is consistent with heating of the shallow part of the water column. Deeper populations of *N. grandis* at about 30 m depth apparently persisted in numbers comparable to those observed in 1968-69, insofar as our limited opportunities to assess them have permitted. A thermal cause is consistent with crinoid decline at sites along the leeward coasts of both Curaçao and Bonaire. Other, as yet unidentified causes for the crinoid decline should not be discounted. For example, the outbreak of the aspergillosis epizootic among sea fans also occurred in the Netherlands Antilles reefs in 1995 (Nugues and Nagelkerken 2006), just before the decline in crinoids was first noted.

As far as we are aware, the decline in reef crinoids in the Netherlands Antilles has not occurred over the broader region of the tropical western Atlantic. Causey (2008) reported that no crinoids were found during a survey in 2001 on Looe Key Reef in the Florida Keys where they had been seen frequently in the 1960s and 1970s; however, crinoids are still seen on reefs of the Dry Tortugas. Meyer photographed both *D. rubiginosa and D. discoidea* on Molasses Reef in the Florida Keys in 1981 (unpublished observations). According to recent reports crinoids are still abundant in such places as St. John, Virgin Islands (C. Rogers, personal communication to DM) and Jamaica (T. J. Goreau, personal communication to DM).

As rheophilic suspension feeders, crinoids may be sensitive to different environmental changes than reef-building corals and other macroinvertebrates. Because crinoids were once among the most common suspension-feeding macroinvertebrates on these reefs, their rapid decline is a cause for concern. Ongoing monitoring of their population fluctuations could provide a different and potentially informative indicator of the overall health of the reef community.

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Spatial and temporal patterns of coral bleaching around Buck Island Reef National Monument, St. Croix, U.S. Virgin Islands

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Abstract. Limited information currently exists on the recovery periods of bleached corals as well as the spatial extent, causative factors, and the overall impact of bleaching on coral reef ecosystems. During October, 2005, widespread coral bleaching was observed within Buck Island Reef National Monument (BUIS) St. Croix, USVI. The bleaching event was preceded by 10 weeks of higher than average water temperatures (28.9-30.1°C). Random transects (100 m^2) over hard bottom habitats (N=94) revealed that approximately 51% of live coral cover was bleached. Nineteen of 23 coral species within 16 genera and two hydrocoral species exhibited signs of bleaching; species-specific bleaching patterns were variable throughout the study area. Coral cover for *Montastraea annularis* and species of the genus *Agaricia* were the most affected, while other species exhibited variability to bleaching. Although a weak but significant negative relationship (r^2=0.10, P=0.0220) was observed, bleaching was evident at all depths (1.5-28 m). Bleaching was spatially autocorrelated (P=0.001) and hot-spot analysis identified a cluster of high bleaching stations northeast of Buck Island. Bleaching was significantly reduced within all depth zones and habitat types upon subsequent monitoring during April (15%) and October (3%) 2006.

Key words: Coral Reef, Coral Bleaching, USVI, Buck Island.

Introduction

During June to October 2005, a significant coral bleaching event took place ranging throughout the Caribbean and the southeastern U.S. Satellite sea surface temperature data indicated that the thermal stress associated with this warm water was the highest experienced during the previous 20 years (NOAA Coral Reef Watch, http://coralreefwatch.noaa.gov/caribbean2005/, accessed 7/02/2008). In October 2005, data on extent and severity of the coral bleaching were recorded during the biannual monitoring of coral reefs in St. Croix, US Virgin Islands conducted by scientists from NOAA's Center for Coastal Monitoring and Assessment's Biogeography Branch (CCMA-BB) and the National Park Service's Buck Island Reef National Monument.

This study quantifies spatial patterns of coral bleaching observed along the northeastern shore of St. Croix comprised of habitats within and adjacent to BUIS and the northern portion of the East End Marine Park (EEMP) during October 2005 through October 2006 (Fig. 1.). Specific objectives include:

1) describing the extent, severity and spatial patterns of coral bleaching before, during and after the 2005 bleaching event
2) correlating coral bleaching with environmental factors such as *in situ* temperature and depth
3) describing taxonomic differences in the severity of bleaching experienced by different coral species.

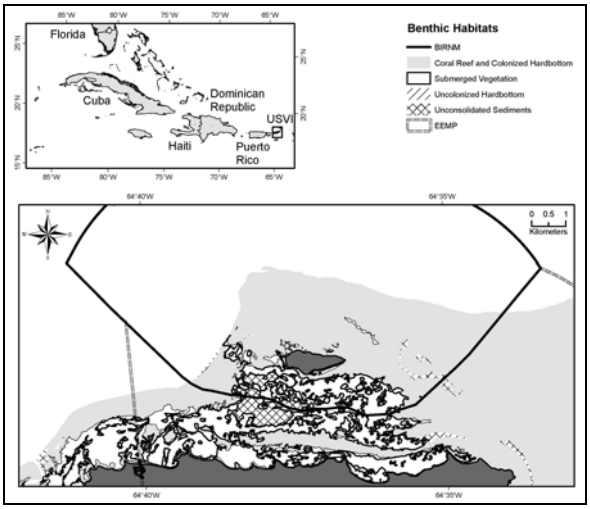

Figure 1: Map of study area around Buck Island Reef National Monument. BIRMN = Buck Island Reef National Monument; EEMP = East End Marine Park.

Material and Methods

Data on benthic composition were recorded from five $1m^2$ quadrats along randomly selected 25×4 m belt transects (100 m^2) as part of a larger project that surveyed 2,790 sites to characterize and monitor fishes and benthic composition in coral reef ecosystems in the US Virgin Island and Puerto Rico between 2001 and 2006. Survey sites were selected using a stratified random sampling design (Menza et al, 2006). Colonies were considered entirely bleached if they contained white, blotchy/mottled, or pale tissue (Fig. 2.). In situ water temperature data were obtained for January 1999 - December 2006 from a single data logger located at 10 m on the fore-reef at BUIS. Linear regression was used to examine the relationship between depths of sites and proportion of total coral cover bleached at each site. Autocorrelation of spatial bleaching patterns was tested using Moran's Index and Geary's C statistic. Time series plots of the proportion of live coral that was 'normal' or 'bleached' from October 2005 through October 2006 examined temporal trends in coral bleaching.

Figure 2: Bleached colony of *Acropora palmata* observed in the study area during October 2005. Quadrat in frame is $1m^2$, with 10cm x 10cm divisions.

Results

From October 2005 through October 2006, 27 scleractinian coral species within 21 genera and two species of fire coral were observed within 294 transects. Water temperatures were consistently higher during the study period when compared with the eight year mean (Fig. 3.).

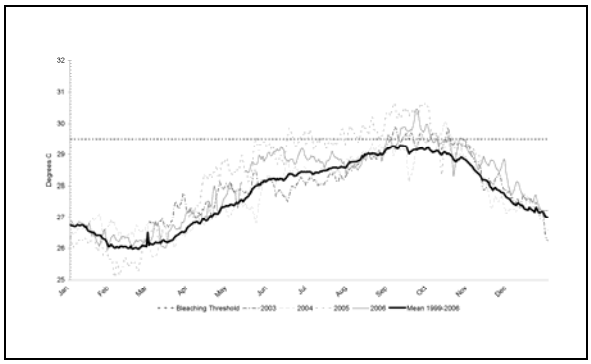

Figure 3: In situ water temperature from the forereef (10m) of BUIS 2003 – 2006. Flat dashed line is bleaching threshold (29.5^0C). Data provided by NPS.

During 2005, mean water temperature was significantly greater ($p<0.0001$) for each summer/fall month (Jun-Oct) than all other years.

October 2005

Mean percent of live scleractinian and fire coral cover (per 100 m^2) ranged from 0.96% on reef rubble to ~ 6% on linear and patch reefs. Surveys (n=94) identified 23 scleractinian coral species (16 genera) and two hydrocoral species. Bleached corals were observed at 91% of all transects (Fig. 4.). Bleached colonies were completely white, with few being mottled or pale. Fifty-one percent of the total coral cover within transects was bleached, and species-specific bleaching proportions ranged from 15.8-100%.

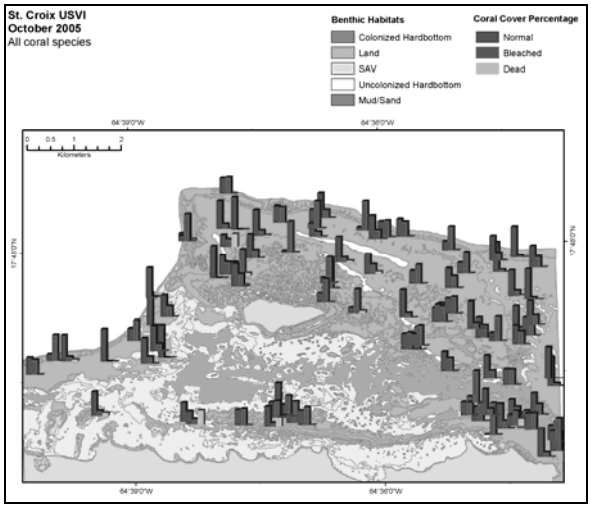

Figure 4: Coral cover percentages of all coral species that were observed as normal (showing no signs of bleaching or discoloration), bleached or dead at each site during October 2005.

April 2006 – Post Bleaching

Bleaching was significantly reduced ($p<0.0001$) throughout the study area during April 2006 vs. October 2005. Surveys (n=89) identified 31 species of

coral where 15% of total coral cover exhibited bleaching (Fig. 5.). Most colonies were mottled/pale, except *Agaricia*, which were completely bleached. Fifteen species exhibited no bleaching affects. Colonies of *M. annularis* and *D. strigosa* were less bleached than those observed in October 2005. *Agaricia* cover was less bleached (16%) than observed in October 2005 (92%). Only two species had bleaching percentages greater than 50% (*Millepora complanata* and *D. labyrinthiformis*) but were infrequently encountered. Overall, nearly all species showed a dramatic decrease in bleached cover within all depth zones.

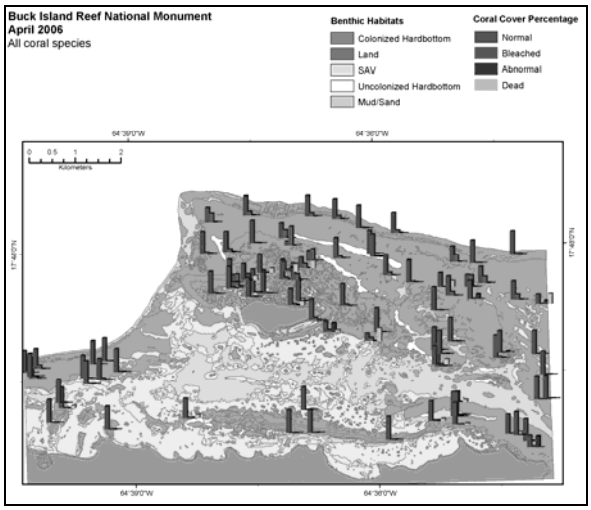

Figure 5: Coral cover percentages of all coral species that were observed as normal (showing no signs of bleaching or discoloration), bleached or dead at each site during April 2006.

October 2006 – One Year Post Bleaching

Bleaching was significantly lower ($p<0.0001$) than during the two previous sampling periods. Surveys (n=93) identified 20 scleractinian coral (5 genera) and two hydrocoral species. Bleaching was evident in 14% of transects with 3% of the total coral cover bleached. Most species exhibited significant declines in bleached cover within all depth zones compared to October 2005 and April 2006 (Fig. 6.). Dramatic decreases were observed for *M. annularis*, *P. asteroides*, *Agaricia*, *P. porites*, and *D. strigosa*.

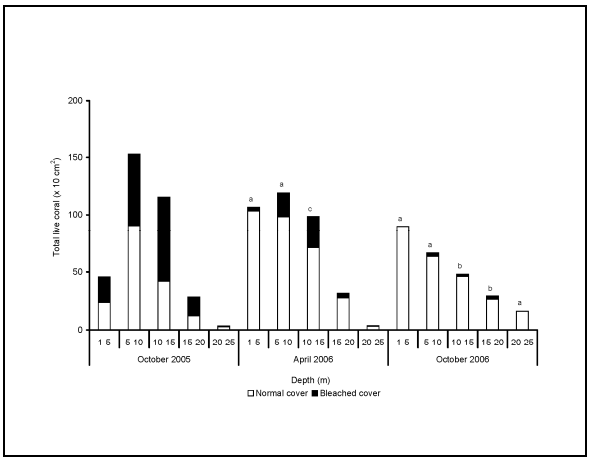

Figure 6: Normal and bleached coral cover within depth zones during study period. a=statistically different from Oct 2005; b=statistically significant from Oct 2005 and Apr 2006; c=statistically significant from Oct 2005 and Oct 2006. All at alpha=0.05.

Discussion

Bleaching events may alter the abundance of functional groups and their component species and have profound effects on the ability of local communities within the ecosystem to resist and recover from future disturbances. A better understanding of these indicators may help managers identify, design and manage protected areas to promote reef ecosystem survival. Results here provide evidence that bleaching has differential effects to the coral community; it is unknown what degree these effects have upon reef community structure. Understanding reef degradation (i.e. coral bleaching) at various scales and the potential for recovery should be a priority for scientifically based conservation and management plans.

Acknowledgement

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Declining Trend on the Mesoamerican Reef System Marine Protected Areas

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Abstract. The Mesoamerican Barrier Reef System (MBRS) comprises coral reefs in Mexico, Belize, Guatemala and Honduras. The Synoptic Monitoring Program (SMP) was developed to assess the coral reefs and associated ecosystems. This program is currently applied in most of the MPAs in the region. It includes sites selected within MPAs that represent strategic sites (Take and No take sites) for park managers on the back reef, shallow (5-12m) and deep fore reef (14-20 m). In 2004, the MBRS region had an average live coral cover of 23%; within a range that is generally considered to be healthy. The maximum coral cover was 50% on deep fore-reef sites, while 2% was the minimum cover at shallow fore-reef sites. The mean cover numbers indicated that the 3 main habitats were relatively healthy. Fish site density averages 34.7 fish per $100m^2$ with ranges from 5.0 to 110.6 ind./$100m^2$. The coral cover had decreased from 23 to 13% in only 4 years in the MPAs, the most dramatic loss of coral was in 2005, with an average loss of 2% in each of the next 3 years. Although monitoring is not equal in all countries and each MPA had different sample sizes, the MBRS region have a generalized coral cover decrease tendency.

Key words: Mesoamerican Reef, Coral Reef, Coral Cover, Status, Monitoring.

Introduction

The Mesoamerican Barrier Reef System (MBRS) comprises coral reefs in Mexico, Belize, Guatemala and Honduras (Arrivillaga and Garcia 2004); it extends more than 1,000 km in Caribbean waters of those four countries (McField and Kramer 2007) and has been considered one of the greatest biodiversity points in the Caribbean (Roberts et al. 2002). The MBRS contributes to the stabilization and protection of the coasts, maintain coastal water quality and serve as feeding and nursery habitats for many marine organisms which have great commercial importance (Almada-Villela et al. 2003).

Major threats in the region are the destruction of natural coastal habitats by increasing coastal population and tourism developments, and increased sedimentation due to extensive and unsustainable use of watersheds and inland deforestation (Arrivillaga and Garcia 2004). The MBRS Project carried out from 2004 to 2008 as a regional cooperation between Mexico, Belize, Guatemala and Honduras had the objective of developing actions focus on the conservation and sustainable use of the system.

The MBRS Synoptic Monitoring Program (SMP) was developed to standardize the assessment of coral reefs and associated ecosystems in marine protected areas (Almada-Villela et al. 2003). The information is targeted at natural resource managers by providing the status and trends in the marine and coastal resources. This article includes a brief summary of the baseline (2004) data for the Coral Reef Ecosystem Results of the SMP for the region and the Status for 2008.

Material and Methods

The Mesoamerican Barrier Reef System (MBRS), is one of the longest reef developments in the Western Hemisphere (Fig. 1), it extends over 1000 km from

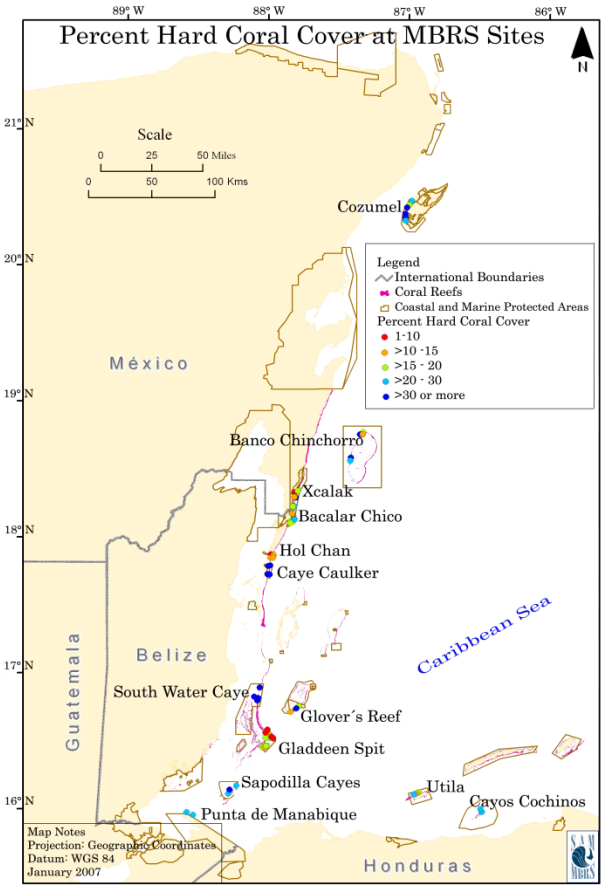

Figure 1. Marine Protected Areas in the Mesoamerican Barrier System included on the SMP. Different color points indicate percent of hard coral cover in each site. See legend on the frame.

Yucatan in Mexico, through Belize to the Bay Islands in Honduras (Mcfield and Kramer 2007).

The Synoptic Monitoring Program (SMP) of the MBRS project was developed based on different methods already used for the region (Almada-Villela et al. 2003). To define sites for the purposes of the SMP, terminology and procedures followed those of Woodley (1999) except that it uses *Location* instead of *Area* (Almada-Villela et al. 2003): Each *Location* contains *Sites* distributed in one or more *Habitats*: shallow back-reef (1-5 m), shallow fore-reef (5-12 m) and deep-fore reef (14-20 m). Most *Locations* are situated in Marine Protected Areas except for Akumal in Mexico. Sites were selected strategically based on particular interest to local management (i.e. sites for use or non-take zones). The data assessed here includes results of reef monitoring carried out from 2004-2008 at *Location* level in each of the four countries.

From North to South assessed *Locations* were located in Mexico, at reefs of Cozumel, Akumal, Banco Chinchorro and Xcalak; in Belize, at reefs located at Bacalar chico, Hol Chan, Caye Caulker, South Water Caye, Gladen Spit, Sapodilla Caye and Port Honduras; Punta Manabique reef in Guatemala; and Utila, Cayos Cochinos and Sandy Bay reefs in Honduras. Protocol for coral reef monitoring includes three components: a) benthic cover, b) coral colonies condition and c) fish community.

a) Benthic cover was assessed in five 30m transects by point intercept transect method including benthos components in each of the following categories: hard coral (to genera), sponges, algae, calcareous algae, soft coral, other living organisms, rock and sand.
b) Colonies of hard coral are identified to species measuring maximum diameter and height. Condition is evaluated by percentage of recent and old mortality and percentage of diseases, bleaching and other stresses.
c) Methods for fish evaluation are based on the AGRRA protocol. Census of 8 indicator families and selected species (www.agrra.org) are conducted using 8 belt transects of 30x2 m. Individuals are identified to species and classified based on size classes.

Number of *Sites* was variable among *Locations* due to the differences interests and objectives of managers (Table 1). SMP was a regional effort applied in each country depending on the management capacity (logistics, staff, resources, etc.) of each MPA.

In order to establish a first baseline of the status of the MBRS for future comparisons, the average value for the parameters recorded in each location along the MBRS was related to a condition category scaled for each component. After stony corals, algae are considered the most prevalent component of the reef benthic community. So coral and algae cover were divided into five categories based on ecological status (Table 2). These categories have been determined based on their relevance to management followed by general recommended actions.

Results

Baseline 2004-2005

Mexico

In 2004, there was 24% average coral cover in the three MPAs: Xcalak, Banco Chinchorro, and Cozumel Island. The lowest (18%) was in Xcalak with higher algal cover (41%), which indicates 'alert' status, similar to that in Cozumel with less coral cover (27%) than algal cover (32%).

Table 1. Sites monitored and sample size in numbers of transects for the benthic component by MPA.

Country	MPAs	Sites	Maximum number of transects+	2004	2005	2006	2007	2008
Mexico	Akumal	12	60	-	-	5	113*	30
	Banco Chichorro	8	40	37	-	-	34	25
	Cozumel	6	30	30	30	30	30	-
	Xcalak	6	30	30	-	58*	3	-
Belize	Bacalar Chico	5	25	20	22	10	15	-
	Caye Caulker	4	20	20	20	20	16	-
	Gladden Spit	10	50	41	63*	5	47	-
	Glover's Reef	3	15	-	15	-	-	-
	Hol Chan	4	20	-	20	40*	40*	-
	Port Honduras	6	30	10	4	48*	6	-
	Sapodilla Cayes	3	15	15	15	20*	12	-
	South Water Caye	4	20	20	5	-	-	-
Guatemala	Punta Manabique	2	10	10	10	-	-	-
Honduras	Cayos Cochinos	5	25	25	25	25	25	-
	Sandy Bay	5	25	-	-	-	23	-
	Utila Island	3	15	-	15	15-	-	-

+ The numbers of transects were standardized to five by site, because in some localities found more than six.
* Some sites were monitored two times along the year.

However, on Banco Chinchorro conditions were considered to be 'optimal' with less algal abundance (23%) than coral cover (27%). The average diameter of coral colonies from the country (31 cm) was according to the average of the entire MBRS region (33 cm). The most common reef fish species were from Family Acanthuridae, mainly in Banco Chinchorro and Xcalak. Cozumel had the second highest fish densities for the region and Banco Chinchorro have the largest algal grazing (herbivore) population living on the deep fore reef. Akumal started SMP until 2006 so the results were not integrated on the baseline in 2004.

Belize

Average coral coverage was 26%, this coral cover is acceptable in relation to the average of the entire MBRS region from 23 to 26% (García-Salgado et al. 2006; Kramer 2003). Although the country has a general 'good' status, three MPAs had more than 40% algal cover and lowest coral cover: Bacalar Chico was considered to be 'alert' status (less than 19% of coral cover), Hol Chan and Gladden Spit were in a 'poor' condition (11% of coral cover). The diameter of coral colonies, however, was greater than in all other countries (maximum 70 cm). The lowest populations of coral reef fishes were found in Caye Caulker and Gladden Spit; whereas Bacalar Chico Location had the most abundant species of herbivores (Acanthuridae, Scaridae) on the deep fore reef.

Guatemala

Only two sites in the Punta de Manabique MPA were monitored. The small reef patches had an average coverage of 26% with the sediment-resistant species (*Montastraea cavernosa, Siderastrea siderea, Agaricia* spp.) being most common. These reefs patches experience high sedimentation and river discharge that impedes coral growth, and possibly also reduces algal growth due to poor light penetration; low algal cover of 25% was measured according to the average of the entire MBRS region (35%). These patch reefs may serve as reference sites as the first reefs to be affected by human activities, such as sedimentation, nutrient loading, harmful chemicals, and solid waste.

Honduras

Utila Island and Cayos Cochinos Locations include similar reef structures as island platforms with an average depth of 5 m, culminating in a steep slope. Utila Island has low coral coverage (16%) and is considered to be in 'alert' status, whereas Cayos Cochinos was in good condition with 24% average coral cover. This Location had the most abundant number of species of herbivore fishes (Scaridae, Acanthuridae) from the locations evaluated in Honduras, however, both Locations (Cayo Cochinos and Honduras) have a critical level of algal cover (> 40%).

Status 2007- 2008

Although monitoring is not equal in all countries and each MPA had different sample sizes, on average coral cover has decreased by 10% in only 4 years. The year with the highest decline in coral cover was 2004-2005 with a maximum of lost in Mexico (11%) and an average in the MBRS region of 7%. In the next years the coral cover show a great variability between MPAs, even though throughout the region had an average loss of 2% (Fig. 2).

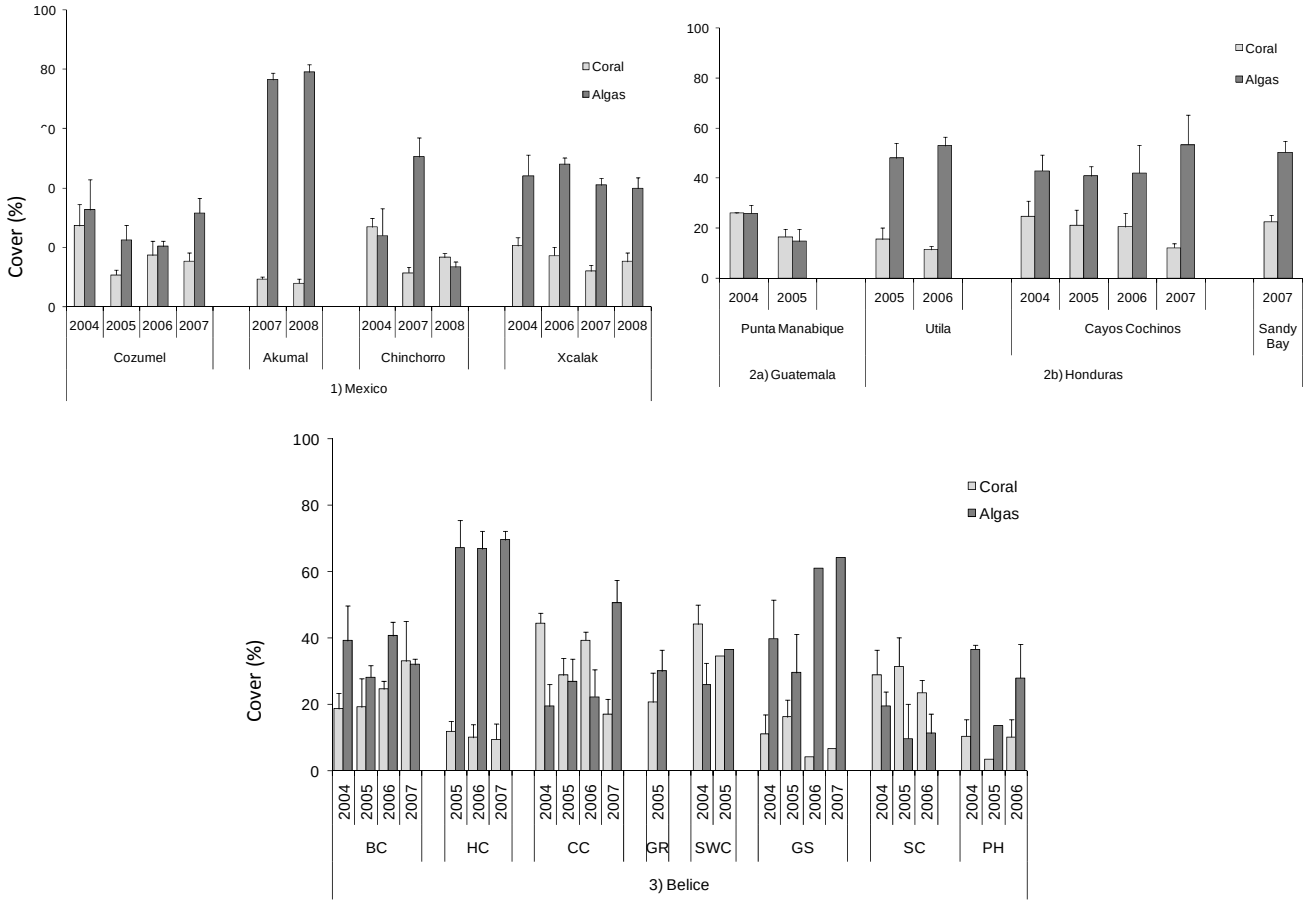

Figure 2: Coral and algal coverage trends in the MBRS with standard deviation. 1) Mexico, 2a) Guatemala, 2b) Honduras and 3) Belize (BC, Bacalar Chico; HC, Hol Chan; CC, Caye Caulker; GR, Glovers reef; SWC, South Water Caye; GS, Gladen Spit; SC, Sapodilla Caye and PH, Port Honduras).

Mexico

All MPAs in Mexico report a decrease in coral cover and an increase of algal cover. Reefs in the region were damaged by hurricanes Emily and Wilma in 2005, Beta and Gamma storms at the end of the same year and hurricane Dean in 2007 with direct loss of live coral cover and variations in fish densities. Parallel, increases in algal cover were variable; for example, in Banco Chinchorro the algal cover increased from 24% in 2004 to 50% in 2007 and then dropped to 15% in 2008, possibly as to physical removal from Hurricane Dean in 2007; as reported in other hurricanes (Lapointe *et al.* 2006). The Akumal Location was added to the monitoring program in 2006 and the first surveys showed low coral cover (mean 10%) and high algal cover (mean 75%); so it was considered to be in a 'critical' status.

Belize

Coral cover in Bacalar Chico MPA had increased by 14% (in 2004 the coral cover was 18% and in 2008 was 33%), with fluctuations in the algal coverage. In 2004 this site was considered as 'alert' status; by 2008 it was regarded as being in good condition. The other MPAs in Belize showed a generalized decrease tendency in coral cover and an increase in algal cover. Unfortunately, Hol Chan and Gladden Spit were regarded as being in a 'critical' condition because these MPAs had less than 10% of coral cover and more than 60% of algal cover.

Guatemala

Monitoring in Guatemala had covered only 2 years, with a same tendency for coral and algal coverage to decrease from 26% and 25% to 16% and 14%, in each component. These tendencies showed a generalized loss of biotic component cover of reefs ecosystem.

Honduras

The Sandy Bay MPA was added to the monitoring program in 2007 with coral cover of 22% and algal cover of 50%. Utila and Cayos Cochinos showed a decrease tendency in coral cover. On Cayos Cochinos

Table 2. Scale for condition category in algae and coral components for MBRS locations. Cover percentage limits for each of the five categories: Critical, poor, alert, good and optimal.

Category	Coral Cover		Algae Cover		Action recommended
	From (%)	To (%)	From (%)	To (%)	
Critical	1	10	>50	-	Stress agents must be determined in order to propose mitigation and/or restoration actions. These sites will also require more effficient management and the feasibility of closing the site to all types of activities must be considered.
Poor	10.01	15	40.01	50	
Alert	15.01	20	30.01	40	Possible stress agents (pollution, nutrient loading, herbivorous decreased) need to be determined in order to propose preentive actions and avoid deterioration
Good	20.01	30	20.01	30	Continue monitoring to detect possible changes on coral and algae cover correlations, live components should be surveyed and herbivores fish protected to keep algal growth low in coverage.
Optimal	>30.01	-	1	20	

the coral cover dropped by approximately 50%, in 2004 it was 24%, and in 2008 the cover was only 12%. Meanwhile in Utila the decrease of coral cover was from 15 to 11%.

Status of fish community

Fish monitoring showed temporal variation between the two semesters of the year (Table 2) along the MBRS region. In Mexico (Fig. 3), Akumal, which lacks of a legal protection, did not have significant changes since 2006, but had the lowest density below the baseline regional average registered in 2004. Banco Chinchorro maintained high values for fish abundance, with the higher densities of families Acanthuridae, Scaridae and Serranidae. In Cozumel, immediately after Hurricanes Emily and Wilma (2005-2) densities reached far beyond the average (72 ind/100m^2) probably due to a temporary redistribution of individuals into non-damaged habitats (Lassig 1983; Walsh 1993), changes in feeding sites and a higher activity of the individuals (Kaufman 1983) as well as for the presence of large carnivores possibly due to redistribution of habitats (Kaufman 1983; Letorneur *et al*. 1993). The effects of the storms were observable until 2006 with a reduction in fish abundance. However, the following year (2007) an increase in density could be observed for families Haemulidae and Pomacentridae.

Protection on the National Park in Xcalak had a positive effect on fish populations according to the baseline data from 2004 to 2006 which could be a consequence from the application of the management program in 2004. A similar situation to that in Cozumel appeared to occur in Xcalak after hurricane Dean (2007-2) which affected the southern most part of Quintana Roo. Density increased above the average (60 ind/100m^2) followed by a considerable reduction in the first period of 2008 (44 ind/100m^2). Values were still above the regional average but carnivores (Serranidae and Lutjanidae) remained with low values.

Gladen Spit and Caye Caulker in Belize (Fig. 3) had the lowest densities and appear with no significant changes in 2007. Nevertheless in Bacalar Chico fish abundance kept values above the average during 2004, 2005, 2006, dropping in 2007 less than the baseline average (22 ind/100m^2). Hol Chan and Sapodilla Caye were localities in Belize with the higher densities in 2007 (77 ind/100m^2). These reefs showed seasonal variations throughout the years with a major decline in fish abundance during the second period of 2006. This reduction was probably due to the effect of 2005 hurricanes and 2006 first tropical storms in the region but then a considerable increase was noted the following year (2007). Cayo Cochinos in Honduras showed a decreasing trend from 2004 to 2007. Other sites of Honduras could not get enough data for temporal comparison.

Discussion

The MPAs evaluated in the MBRS region shows a generalized trend of decrease coral cover. The most evident loss was reported in the 2004-2005 years (7%); in the subsequent years these tendencies are in the range of 2%. These decreases were largely measured in localities under protection status, which may then represent a more serious coral loss throughout the whole region. The Healthy Reefs Initiative (2008) have shown that even MPA sites monitored here had higher reef health (especially higher coral cover) than the reef in general as measure by the 332 sites in the Report Card.

The results in the SMP in sites under protection status showed effects of perturbation. It seems that strategies have apparently not been sufficient for buffering

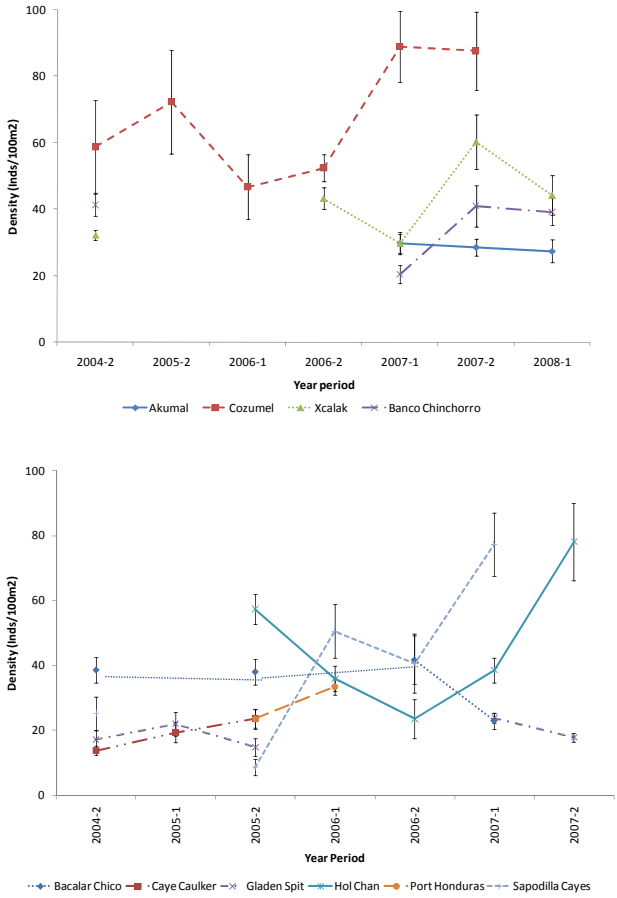

Figure 3: Average fish density (Inds/100m^2) and standard error bars by year (2004-2008) and semester (1-2), in Mexico (upper) and Belize (lower).

the different and constant types of impacts in the region like hurricanes and local structural modifications in the resources as well as conditions in the whole coral reef ecosystem (Nava-Martínez y Alvarez-Filip 2005; Wilkinson and Souter 2008). Thus management strategies have apparently not been the sufficiently effective to attenuate the increasing negative impacts in the reefs. While some of these impacts were natural and therefore difficult to predict, damaging human activities are strongly implicated. The management strategies will have to improve and adopt the precaution principle to mitigate damaging human activities and to improve resilience against natural impacts. Fish resources are coming under greater pressures from increasing tourist development with some species, mainly groupers (Serranidae), snappers, grunts and jacks being targeted by the local fishermen. Lobsters and conch are also very valuable fishery resources, but information is insufficient for effective management. More research and monitoring on the trends in fishing activities in the MBRS region are required.

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Nitrogen fixation in coral reef environments

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Abstract. Coral reefs are sites of high nitrogen fixation activity where marine cyanobacteria are the major contributors. In coral reefs cyanobacteria can be found in very diverse environments: water column, sandy bottoms, on coral rubbles as endolithic and epilithic forms, or forming microbial mats. The purpose of the present research is to evaluate N_2 fixation rates in different environments (sandy bottom, coral rubble and cyanobacteria mats) and their contribution to the net primary production. Two different fringing coral reef sites were studied: Sesoko at Okinawa, Japan and La Reunion at the Indian Ocean. N_2 fixation and primary production rates were measured using ^{13}C and ^{15}N fixation techniques. Daily N_2 fixation in sandy bottom was about 3 nanomoles N/µg Chl-*a*, with near 6% contribution to the primary production. In coral rubbles it ranged from 0.5 to 7 nanomoles N/µg Chl-*a* with 2.4 to 28% contribution to primary production, while in cyanobacteria mats it varied between 0.2 to 242 nanomoles N/µg Chl-*a* with contributions of 2.8 to 42% to the primary production. Differences in N_2 fixation rates between daytime and nighttime indicated the presence of both heterocystous possessing and non-heterocystous possessing cyanobacteria.

Key words: N_2 fixation, Cyanobacteria, Endolithic algae.

Introduction

Biological nitrogen fixation is a process unique to prokaryotes, occurring commonly in freshwater and marine cyanobacteria. Although energetically demanding, this process provides the organisms with a particular advantage when growing under N-limited conditions, which are most frequent in marine environments (Staal et al. 2001). Due to oxygen sensitivity of the nitrogenase enzyme, the fixation of carbon and nitrogen in an oxygen-producing organism needs to be separated either in space or in time (Berman-Frank et al. 2003). Spatial separation is achieved in heterocystous cyanobacteria which are able to fix nitrogen during the day, contemporaneous with their energy generation. Non-heterocystous cyanobacteria temporally separate nitrogen fixation and oxygenic photosynthesis by fixing nitrogen at night, using photosynthetic energy generated during the previous day (Lundgren et al. 2003). Biological nitrogen fixation appears to make a major contribution to N supply in coral reef ecosystems, as has been shown for the Eniwetok Atoll (Webb et al. 1975), the Great Barrier Reef (Larkum et al. 1988), and for the lagoons of Tikehau Atoll (Charpy-Roubaud et al. 2001) and New Caledonia (Charpy et al. 2007). Many studies on N_2 fixation have dealt with shallow coral reefs (Larkum et al. 1988; Shashar et al. 1994, Charpy-Roubaud and Larkum 2005), however they did not identified N_2 fixers in coral rubbles. In the present study, we measured ^{15}N and ^{13}C uptake in benthic cyanobacterial populations associated to coral rubbles as endolithic or epilithic forms, in sandy bottoms, and forming microbial mats in shallow coral reef lagoons at St. Gilles (La Reunion Island, Western Indian Ocean) and Sesoko (Okinawa Island, Japan).

The objectives were to identify *in situ* dominant nitrogen fixers among benthic cyanobacteria, to measure their nitrogen fixation rates and the percentage of new production achieved throughout the N_2 fixation. Moreover we compared the results obtained from two geographically remote sites of Indian Ocean and Pacific Ocean respectively.

Material and Methods

The present study is based on the field research carried out at La Reunion Island in November, 2004 and in February, 2007 and at Sesoko Island of Okinawa in July, 2005 and October, 2006. La Reunion Island is located in the South-Western Indian Ocean (21°7'S, 55°32'E), 700 km east of Madagascar.

The common reef geomorphology includes an outer slope, outer and inner reef flats and a back reef. The fringing reefs are discontinuous and narrow covering only 7.3 km^2. The reef flats are exposed at low tide. The studied area was located at La Saline along the southwestern coast of Reunion Island. The depth of the study area never exceeded 2m. Live coral coverage varied between 20 and 30% and macroalgal cover reached 40% on the reef flat. Sesoko Island (26°38'N, 27°51'E) is located off the NW coast of Okinawa in the Ryukyu Archipelago, South-Western Japan. The sea around the Ryukyus is under the influence of the warm Kuroshio Current and characterized by the development of coral reefs from latitudes 24° to 31°N, marking their northernmost distribution. Two sampling stations were selected in July, 2005 and October 2006, Station B (1 to 2m depth) located in a moat on the west coast of Sesoko Island behind a well-developed reef crest, located 150–200 m from the shore, and Station C (2 to 3m depth) located next to the pier of Sesoko Station, Tropical Biosphere Research Center of the University of the Ryukyus. Reefs around Sesoko Island are actually in a post bleaching phase with less than 10% of living coral coverage, 50% of sandy bottom, 30% of coral rubbles and the rest covered by macroalgae and turf algae (Casareto, unpublished data).

Temperature and light were monitored over the duration of the surveys using in situ sensors (MDS-MkV/T and MDS-MkV/L, Alec electronics). Salinity and nutrient concentrations were routinely measured during field studies. Triplicate sub-samples of sea water for nutrient measurement were collected into clean acid-washed 100 ml polyethylene bottles and kept frozen. Nutrients were determined with an autoanalyzer (TRAACS-2000: BRAN+LUBE) according to Hansen and Koroleff (1999). Nitrate was determined by subtracting the values of nitrite from nitrate + nitrite. The detection limits were 0.052 µM for NO_3 + NO_2, 0.01 µM for NO_2, 0.020 µM for NH_4 and 0.020 µM for PO_4. Reproducibility of nutrient analysis was ± 0.5% .

Sampling of coral rubbles, sand and cyanobacteria mats were done using snorkeling or scuba diving, and documented by underwater photography. Samples were gentle placed into sterilized tubes or plastic bags, kept in cool box and brought back to the laboratory for sub-sampling and treatments. In the laboratory aliquots of the materials were placed in small flasks and kept with formaldehyde (4%) for later microscopic analysis and taxonomic identification. Other aliquots were used in incubation experiments for N_2 fixation and primary production measurements. For coral rubbles, two small branches of similar size were placed into the incubation bottle which was filled with seawater collected simultaneously at the same sampling point (incubation of epilithic plus endolithic algae). Other two branches of similar size were previously gentle brushed to remove the epilithic community and place in another incubation bottle also filled with seawater of the sampling point (incubation of endolithic algae). Aliquots of 10g of sand were placed into the incubation bottles which were filled with seawater from the same sampling point. Aliquots of approximately $1cm^2$ of cyanobacteria mats were placed into the incubation bottles and filled with seawater from the same sampling point. Control incubations using only seawater from the sampling points were carried out. All incubation bottles were enriched with ^{15}N and ^{13}C, and incubated *in situ*. Incubation started before the sunset and continued until early morning (12h incubations) for N_2 fixation of non-heterocystous cyanobacteria. For heterocystous cyanobacteria the incubation continued until the end of the following day (Charpy et al. 2007).

N_2 fixation rate and dissolved inorganic carbon uptake measurements were carried out according to Slawyk et al. (1977): Samples placed into polycarbonateincubation bottles of 180 mL fitted with a septum, were enriched with 0.36 mL of ^{13}C-labelled sodium bicarbonate ($NaH^{13}CO_3$ – 100mg in 10 mL of deionized water – 99.9% ^{13}C) to obtain an enrichment of 11.5%. Subsequently, 0.36 mL of $^{15}N_2$ (99.8 atom %, Shoko Co. Ltd, Tokyo, JAPAN) was added with a gas-tight syringe to obtain an enrichment of 6.8%. For mats, incubations were terminated by filtration under gentle pressure through a precombusted 47-mm diameter GF/F filter, acidified by HCl fumes and dried. For coral rubbles and sand, small subsamples were mashed on mortars and treated with HCl to remove the carbonates, and posterior filtrated onto precombusted 47-mm diameter GF/F filter. Filters were dried on 60°C for POC, PON, and isotope analysis. Similar subsamples without acidification treatment were prepared for HPLC analysis. Measurements of delta ^{13}C, delta ^{15}N, and POC, PON were done using a mass spectrometer DELTA plus Advantage (Thermofinigan Co.) equipped with EA1110 for measurements of POC and PON. Primary production was calculated according to Hama et al. (1993) and N_2 fixation rate was calculated by isotope mass balance as described in Montoya et al. (1996). Chlorophyll (Chl-*a*) analyses were performed by spectrophotometry for La Reunion 2007 samples and by HPLC for La Reunion 2004 and Sesoko samples. Chlorophyll for spectrophotometric analysis was extracted in 10 ml of 100% methanol, and optical density at 665 nm was recorded before and after acidification. Chl-*a* concentration was calculated using the extinction coefficients given by Porra et al. (1989). For HPLC analysis, the pigments were

extracted with 95% ethanol. HPLC was carried out with a model LC-10AT$_{VP}$ (Shimadzu, Kyoto, Japan). Pigments were eluted at a flow rate of 1.0 mL/min at 25°C with a programmed binary gradient elution system. Separated pigments were detected spectrophotometrically with a photodiode array detector (Shimadzu SPD-M10AVP), measuring from 400 to 760 nm.

Results and discussion

Environmental parameters

Temperature, salinity and nutrient concentrations in the ambient water of studied sites at La Reunion and Sesoko islands are summarized in Table 1. The total dissolved inorganic nitrogen (DIN) was highest in La Reunion in November 2004 (1.71 µM) and lowest in Sesoko in Station B in October 2006 (0.20 µM). In La Reunion, high values of ammonia and nitrate indicate some degree of eutrophication and coastal pollution. Reduced nitrogen compounds were always low in Sesoko. Dissolved PO_4 concentrations were low (<0.21 µM) in all stations during all the study period. Water temperature and salinity in both sites were comparable during the study period and so was the photon flux.

Table 1. Environmental parameters

Place	T °C	Salinity	Light intensity	Nutrients (µM)				
			(µmol cm^{-2} sec^{-1})	NO_3	NO_2	NH_4	DIN	PO_4
La Reunion								
Nov. 2004	28.5	32.3	1928 ± 854	0.45	0.26	1	1.71	0.1
Feb. 2007	26.3	35.1	1696 ± 724	0.32	0.13	0.1	0.55	0.21
Sesoko								
St. B July 2005	29.4	34.3	2104 ± 497	0.66	0.04	0.1	0.8	0.07
St. B Oct. 2006	27.6	34.5	1816 ± 598	0.09	0	0.11	0.20	0.14
St. D Oct. 2006	27.3	34.6	1442 ± 392	0.28	0.04	0.17	0.49	0.04

Taxonomy

Algae associated to coral rubbles were studied microscopically after gentle brushing of rubbles previously fixed using formaldehyde. For the endolithes gentle acidification treatment was done. Algae associated with sand or cyanobacteria forming mats were studied under microscope from formaldehyde fixed samples. The epilithic algae were composed by a wide spectrum of algae including chlorophytes, phaeophytes, rhodophytes and bacillariophytes. Cyanobacteria of the genus *Lyngbya, Oscillatoria* and *Phormidium* were the main as epilithic in La Reunion and *Calothrix, Gardnerula* (both possessing heterocysts), *Hydrocoleum* and *Lyngbya* in Sesoko. Endolithic algae were composed by chlorophytes (mainly *Gomontia* and *Ostreobium*) and *Hyella* (cf.) *caespitosa*, *Plectonema* (cf.) *terabrans*, *Mastigocoleus testarumin* and *Scytonema* (cf.) *conchophillum* (the last two species with heterocysts). Same species of cyanobacteria and a high diversity of epilithic diatoms were found in sandy bottoms at both sites. Among the cyanobacterial mats the main identified species in La Reunion were *Nodularia* (with heterocysts*)*, *Oscillatoria* and *Leptolyngbya* at La Reunion and *Hydrocoleum coccineum*, *Nodularia harveyana* (with heterocysts) and *Phormidium laysanense* at Sesoko.

N_2 fixation rates in coral rubble

N_2 fixation rates in coral rubbles from La Reunion and Sesoko at different studied seasons are shown in Table 2. At La Reunion, rates for the incubations with only the endolithic alga during the first 12h (dark period) varied between 0.4 to 0.9 nanomoles N per µg Chl-*a*, and were 2.8 to 6.8 nanomoles N per µg Chl-*a* during 24h incubation. This shows that N_2 fixation was performed mainly during the 12h light period, indicating that heterocysts possessing cyanobacteria were the main components of endolithic algae. N_2 fixation rates for rubble incubations with endolithic plus epilithic algae varied from 0.33 to 2.7 nanomoles N per µg Chl-*a* during 12h (dark) and 0.45 to 4 N per µg Chl-*a* during 24h of incubations. These results show that the contribution of epilithic algae to the total N_2 fixation in rubbles is not so important if compared with rates obtained for only endolithes. Moreover in November 2004 N_2 fixation rate during 24h for endolithic plus epilithic algae was lower than that during 12h dark period, indicating that during day time there is a loose of N_2 fixed during the night. For the overall data of coral rubble in La Reunion Island it seems that N_2 fixation raters are higher in February (summer) than that of November (dry season). At Sesoko, N_2 fixation rates of endolithic alga during the first 12h (dark) incubations varied between 0.6 to 3.6 nanomoles N per µg Chl-*a* and 1.4 to 7.2 nanomoles N per µg Chl-*a* during 24h. Higher values of N_2 fixation during 24h incubations indicate that during light period N_2 was fixed and therefore this shows the presence of heterocyst possessing cyanobacteria. Rates for incubation with endolithic plus epilithic varied from 1.9 to 5.5 nanomoles N per µg Chl-*a* during the 12h dark period and 0.8 to 1.4 nanomoles N per µg Chl-*a* during 24h. These results show that in incubations with both epilithic plus endolithic algae fixed N_2 during the night was lost during the light period. For the overall data at Sesoko it seems that N_2 fixation rates by coral rubbles are higher in October (post summer season) than that of May (spring).

Table 2. Primary production, N_2 fixation rates and the contribution of N_2 fixation to net primary production of coral rubbles at La Reunion and Sesoko Islands. En: Endolithic algae; Ep: Epilitic algae

Site	Organism Rubble	Primary Production (nmoles C µg Chl-a^{-1}day^{-1})	N_2 fixation 12h (dark) (nmoles N µg Chl-a^{-1} time^{-1})	N_2 fixation 24h (nmoles N µg Chl-a^{-1} time^{-1})	C/N	Organic carbon prod. by N_2 fixation (nmoles C µg Chl-a^{-1}day^{-1})	Contribution of N_2 fix. to Primary Production (%)
La Reunion (Feb. 2007)	En	194.7 ± 53.8	0.9 ± 0.1	6.8 ± 2.3	6.9 ± 0.3	46.6 ± 13.7	23.8 ± 0.5
	Ep + En	191.91 ± 34.1	0.33 ± 0.2	4.0 ± 0.4	7.9 ± 0.8	32.0 ± 6.8	16.6 ± 0.6
La Reunion (Nov. 2004)	En	399.4 ± 300.1	0.4 ± 0.2	2.8 ± 1.3	21.6±11.7	76.1 ± 55.5	28.1 ± 11.7
	Ep + En	210.62 ± 42.5	2.7 ± 1.8	0.45 ± 0.18	11.6±6.1	5.5 ± 3.5	2.4 ± 1.1 n = 12
Sesoko (May 2007)	En	356.2 ± 271.1	0.6 ± 0.4	1.4 ± 0.52	9.0 ± 2.4	12.6 ± 6.8	3.5 ± 0.2
	Ep + En	134.6 ± 55.2	1.9 ± 0.4	1.43 ± 0.5	9.7 ± 0.6	13.8 ± 5.2	10.3 ± 0.3
Sesoko (Oct. 2007)	En	366.6 ± 6.1	3.6 ± 0.6	7.17 ± 0.9	8.5 ± 0.5	60.9 ± 5.2	16.62
	Ep + En	176.2 ± 84.2	5.5 ± 3.8	0.77 ± 0.4	9.6 ± 1.1	7.4 ± 9.7	4.19 n = 12

N_2 fixation rates of cyanobacteria mats

N_2 fixation rates of cyanobacrteria mats are shown in Table 3. Among the three species of cyanobacteria forming mats *Nodularia* showed high fixation rates (112 nanonoles N per µg Chl-*a*) during 24 hours compared with rates at dark period indicating the efficiency of the heterocyst possessing cyanobacteria. The other two identified species forming mats, *Oscillatoria* and *Leptolyngbia* showed almost no N_2 fixation activity during the day time. In Sesoko *Hydrocoleum coccineum* showed the higher rates of N_2 among all the studied cyanobacterial mats, being 288 nanomoles N per µg Chl-*a* during the night and 242 nanomoles N per µg Chl-*a* during 24h.While these species do not posses heterocysts and the N_2 fixation activity was observed mainly during the night period, these rates were one order of magnitude higher if compared with other mats from Sesoko or two times to two orders of magnitude higher when compare with fixation rates of mats at La Reunion Island

N_2 fixation rates of sandy bottom

N_2 fixation measurements of sand were performed only at Sesoko Island. Rates are shown in Table 4. During the dark period, rates were 0.35 nanomoles N per µg Chl-*a* and 2.8 nanomoles N per µg Chl-*a* in 24 h. These rates are comparable to the lower rates observed for coral rubbles at La Reunion during November.

Table 3. Primary production, N_2 fixation rates and the contribution of N_2 fixation for the net primary production of cyanobacteria mats at La Reunion and Sesoko Islands

Site	Organism	Primary Production (nmoles C µg Chl-a^{-1}day^{-1})	N_2 fixation 12h (dark) (nmoles N µg Chl-a^{-1}time^{-1})	N_2 fixation 24h (nmoles N µg Chl-a^{-1}time^{-1})	C/N	Organic carbon production by N_2 fixation (nmoles C µg Chl-a^{-1}day^{-1})	Contribution of N_2 fixation to Primary Production (%)
La Reunion (2004, 2007)	*Nodularia**	2359.9	0.63	112.2	8.8	987.0	41.8
	Oscilatoria	2063.3	7.80	4.2	13.6	57.0	2.8
	Leptolyngbia	14.0	0.64	0.2	12.6	2.9	20.7
Sesoko (Stn. C)	*Hydrocoleum coccineum*	9125.6	288.1	242.0	10	2419.5	26.5
(Stn. B)	*Nodularia harveyana**	3884.9	0.7	28.5	16	464.7	12
(Stn. B)	*Phormidium laysanense*	6289.3	28.9	27.1	23	629.2	10

*posess heterocysts

Table 4. Primary production, N_2 fixation rates and the contribution of N_2 fixation for the net primary production of sandy bottom at Sesoko Island

Site and 0rganism	Primary Production (nmoles C µg Chl-a^{-1}day^{-1})	N_2 fixation 12h (dark) (nmoles N µg Chl-a^{-1}day^{-1})	N_2 fixation 24h (nmoles N µg Chl-a^{-1}day^{-1})	C/N	Organic carbon production by N_2 fixation (nmoles C µg Chl-a^{-1} day^{-1})	Contribution of N_2 fixation to Primary Production (%)
Sesoko Sandy bottom	545.4 ± 118.2	0.35 ± 0.3	2.85 ± 2.0	10.9 ± 1.6	31.6 ± 25.2	5.7 ± 3.9 n = 6

Contribution of N_2 fixation to primary production:
On the bases of measured C/N ratios of all the studies substrata (see tables 2, 3 and 4) it is possible to calculate the amount of organic carbon which can be fixed as new production on the bases of the fixed atmospheric nitrogen. Further, using the measured primary production rates, it is possible to estimate the contribution of new production to the total primary production of the studied substrata. For coral rubble from La Reunion this contribution varied from 24% to 28% for endolithic algae and from 2.4% to 17% for epilithic plus endolithic algae. At Sesoko the contribution of N_2 fixation to primary production of coral rubbles was lower and varied between 3.5% to 17% for endolithic algae and 4% to 10% in case of epilithic plus endolithic algae. In case of cyanobacteria mats, the contribution of N_2 fixation to the total primary production varied from 2.8% to 42% at La Reunion and from 12 to 28.5 at Sesoko. In sandy bottom, the contribution of new production to the total primary production was 5.7%

Table 5. N_2 fixation rates during night and 24h of coral rubble, cyanobacteria mats and sandy bottom in comparable units of mg N m^{-2} $time^{-1}$

Type of Sediment	Location	N_2 fixation 12h (dark) mg N m^{-2} $time^{-1}$	N_2 fixation 24h mg N m^{-2} $time^{-1}$
Coral gravel	Sesoko	1.45 ± 0.84	2.37 ± 1.93
	La Reunion	0.57 ± 0.37	2.07 ± 1.2
Cyanobacteria mat	Sesoko	64.14 ± 3.05	94.81 ± 7.42
	La Reunion	27.12 ± 7.32	96.98 ± 2.28
Sand	Sesoko	0.20 ± 0.17	3.08 ± 1.79

Conclusions
Biological N_2 fixation was found in all the studied substrata. The presence of heterocysts possessing cyanobacteria was important among the endolithic algae and showed higher efficiencies of N_2 fixation and important contribution to primary production (up to 28%). Highest N_2 fixation was observed in cyanobacterial mats. In particular *Hydrocoleum coccineum* (Sesoko) was the highest among the non-heterocysts cyanobacteria and *Nodularia* (La Reunion) among heterocysts possessing ones. The contribution to primary production was up to 34%. In sandy bottom N_2 fixation rates was similar to that of coral gravel at the same location and season (Sesoko, May), but this fixation represented less than 6% of required N for the primary production.

Table 5 shows a summary of the results of N_2 fixation rates for the three studies substrata in terms of mg of fixed N_2 per m^2. These results show that N_2 fixation rates of cyanobacteria mats are order of magnitude higher than that of coral rubbles or sandy bottom. However, considering the covered area in the whole reef, the contribution of coral gravel and sandy bottom might be the highest. In the present study all cyanobacteria mats were found to be of small size and covered less than 1% of the total reef area.

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Role of organic matter in chemical symbiosis at coral reefs: release of organic nitorgen and amino acids under heat stress

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Abstract. Coral Reefs are declining worldwide due to bleaching and diseases. Biogeochemical role and mechanisms of behavior of dissolved organic matter remain unexplained on this context. Here we discuss the changes of dissolved organic nitrogen by composition of amino acids as a model study, to demonstrate linkage of coral stress to elevated temperature and behavior of chemical substances in the coral biological system. Results suggest that high concentrations of nutrients and organic matters are due to the materials release by coral organism under the elevation of water temperature. This indicates observed organic nitrogen such as amino acids, peptides, proteins released as response to coral thermal stress. C: N and N: P ratio of coral mucus of thermal stressed and initial shows that thermal stress increases production of organic nitrogen and connected to increase of total amino acid could be peptides or proteins. This is shown by total hydrolyzed amino acids released from coral under the heat stress contains mix of peptides or proteins. However, to determine and confirmed source of organic nitrogen within this context need further experiments integrating microbial sources and processes. We propose to include chemical perspective in the coral biological system by the concept of chemical symbiosis.

Key words: TOC, TON, TOP, Amino Acids, Coral Reefs, Chemical symbiosis.

Introduction

Coral reefs are declining at alarming rate worldwide, bleaching and immerging diseases are recorded for the degradation of coral reefs. Coral bleaching damages of the symbiotic zooxanthellae are presently believed to result largely from elevated temperature of the seawater and the increase of UV radiation (Hoegh-Guldberg 1999, Glynn and Crox 1990, Jokiel and Brown 2004). Mild or moderate bleaching is commonly followed by recovery, but the long-term effects of high temperature of seawater will end in an irreversible bleaching and massive death of corals. As an adverse effect of rising up seawater temperature, coral animal undergo stress, response to this stress, corals release high amounts of organic matters as mucus and ammonia (Krupp 1984). The composition of coral mucus contains variable of macromolecular compounds (Ducklow and Mitchell 1979). This suggests that organic matters in dissolved (DOM) and particulate (POM) forms might play an important role for the fate of corals and coral ecosystem (Suzuki et al. 2000 and Casareto et al. 2000) especially for the resistance of coral under the heat stress. The production of organic matters by corals can act as a mechanism for defense but also it is used in feeding for particles capture strategy. High amount of ammonia in the reefs surrounding waters can enhance the growth of pico-phytoplankton (Casareto et al. 2004, Charpy 2004), which acts as main food source for benthic community. However organic matters may enhance the growth of certain bacteria, which are known to infect corals and induce bleaching and diseases (Kline et al. 2006). Therefore release of organic matter play an important role in biogeochemical cycles of coral reef ecosystem. The knowledge from this prospective is needed further to explain the sources and utilization of organic matter cycling in coral reefs to describe coral stress and health. Dissolved organic nitrogen (DON) is that subset of the dissolved organic matter (DOM) that contains nitrogen. Large part of nitrogen compounds in coral reef waters are found as DON, while inorganic nitrogen concentration is very less in coral reef waters, because coral reef is located in the oligotrophic area. But this is "paradox" for understanding of high productivity and bio-diversity of coral reef. Most of DON is consisted of peptide, protein, amino acids, urea, etc, behavior of these

compounds can influenced by coral stress due to sea temperature rise explained in global warming. DON is a heterogeneous mixture of compounds with various time scales from days to hundred years as refractory substances. A large number of compounds have been identified within the DON pool, including dissolved combined amino acids (DCAA) and dissolved free amino acids (DFAA). When stress has been given coral by increasing water temperature, light intensity and others, coral releases mucus as defense against invasion by bacteria and/or virus. It is well known that most of mucus is accepted as carbohydrate and lipid which are DOC, and less protein (Coffroth 1990). However there are very few reports on the DON and protein in mucus. Here we report the behavior of dissolved organic nitrogen and amino acids concentration; distribution and changes in its characteristics (composition of amino acids and molecular weight) elevated water temperature from field and incubation experiments with suggestions link the coral stress and chemical behavior of substances within coral biological system and reef as a whole.

Material and Methods

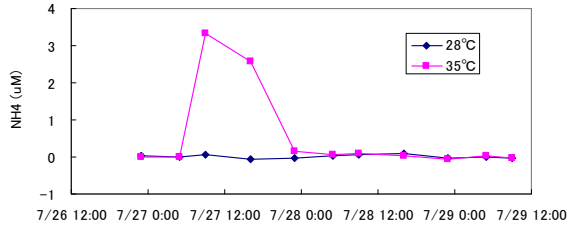
Experiments of heat stress: Experiments were done at the Sesoko Laboratory of Tropical Biosphere Research Center, Ryukyu University, Okinawa, 2006 Japan Fixed 10 branches of *Montipora digitata* specimen collected around Sesoko Island in 400 ml tank. Tanks were filled with filtration water with double filters of GFF and water was circulated with 20 ml $min.^{-1}$ from tank with 100 L to the same tank. All experimental vessels were kept under the water bath with 28^0 and 35^0, respectively. Light intensity was adjusted to 400uM with switch on and off in 12hours interval.

Measurements: Mucus samples for measurements were taken at every day during the times as 5:00, 9:00, 16:00, 23:00, for measurement for TON (total organic nitrogen) and TOP (total organic phosphorus) using an Auto-Analyzer (Braun-Lube) after digestion with alkaline persulfate, nutrients (nitrate, nitrite, ammonia, phosphate) using an Auto-Analyzer (Braun-Lube), amino acids using HPLC(Agilent) and Total Organic Carbon (TOC) by TOC 5000 (Shimadsu).

Results

Variation of inorganic nutrient concentrations recorded for temperature 28^0C and 35 ^{0}C during the experiment (Fig. 1). Also variations of Total Organic Nitrogen (TON) and Total Organic Phosphate (TOP) Concentrations are shown (Fig.2). High concentrations of ammonia, phosphate, TON and TOP were found significantly in samples of 35^0C at 9:00 a.m. of 27, July (Fig.2) Ammonia concentration increased to 3.6μM from 0.04μM with a couple of 100 times. Organic matters concentrations also increased to 29.9μM from3.3μM for TON with 10times and to 0.22μM from0.03μM for TOP.

(a)



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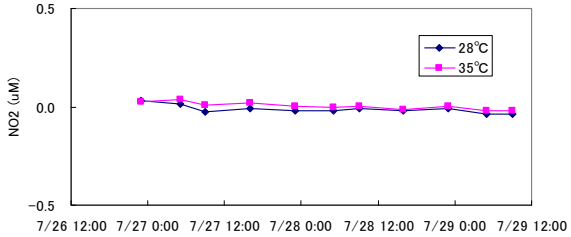

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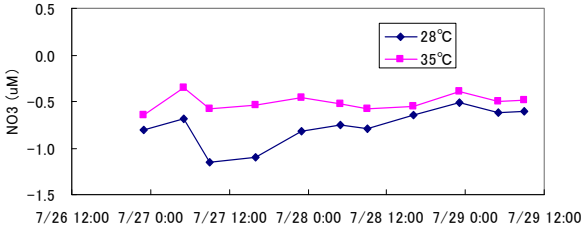

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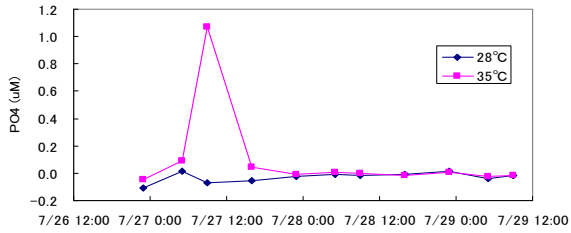
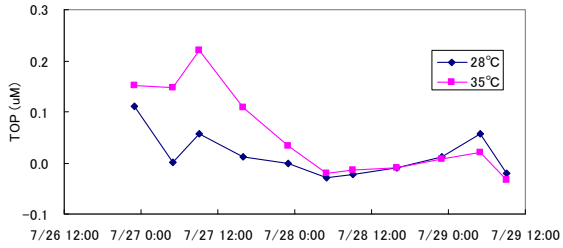


Figure 1: Variation of difference [Δ = (final) - (initial)] in Inorganic nutrient Concentrations (a)Δ Ammonium, (b) Δ Nitrite (c) Δ Nitrate and (d) Δ

Phosphate during the incubation experiments for 28^0C and 35^0C

Table 1 shows change of TOC, TON and TOP concentrations at 35^0 and 28^0C compared with initial concentrations. It was observed that increment of TON (about 10 times) is greater than that of TOC (about 3 times) at 35^0C. In Table 2 shows the Total Hydrolysis combined Amino Acids (THAA) concentrations, % of amino acids for TOC and TON. THAA concentrations increased to 414μg l^{-1}

(a)



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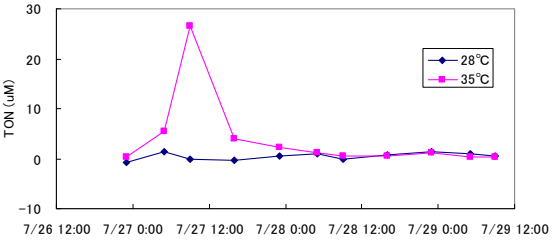

Figure 2: Variation of Concentration for Dissolved Organic (a) Nitrogen and (b) Phosphate during the incubation experiments for 28 ^{0}C and 25^0C

from12μg l^{-1} with 20 times at 35^0, increment rate of THAA was 7.7% for TOC and 13.6% for TON, For understanding characteristics of THAA, we divided into the high molecular weight (more than 1000 Dalton) and low molecular weight (less than 1000 Dalton) using cross flow filtration (ultra filtration techniques) for samples measured THAA. Table 3 shows organic nitrogen and THAA concentration were greater in the high molecular weight fraction for 35^0 than those for 28^0,

Table 1: (a) Mean concentration and standard deviations of Dissolved Organic Carbon and Dissolved Organic Nitrogen and (b) C/N and N/P ratio for the temperatures 28^0C and 35^0C (n=5) with one initial (In)

(a)

Date and Time		Concentration μM TOC	TON	TOP
27 Jul.	28℃	57.7 ±3.4	3.3 ±0.5	0.06 ±0.04
9:00	35℃	187.4 ±56.1	29.9 ±12.6	0.22 ±0.13
	In	60.7	3.4	nd[b]
28 Jul.	28℃	67.2 ±4.4	3.3 ±0.2	0.09 ±0.03
9:00	35℃	66.2 ±3.8	4.0 ±0.4	0.10 ±0.01
	In	59.2	3.4	0.11

(b)

Date	Exp.	Ratio C/N	N/P
27 Jul.	28℃	18 ±2.6	49 ±11
9:00	35℃	7 ±1.5	150 ±58
	In	18	nd
28 Jul.	28℃	20 ±0.7	43 ±20
9:00	35℃	17 ±2.4	41 ±6.7
	In	17	30

a: not detected

Table 2 : Mean concentration changes of Amino Acids and percentages of amino acid carbon to TOC (%AA-C) and amino acid nitrogen to TON for the temperatures 28^0C and 35^0C (n=5) with one initial (In)

Date, Time and Conditon		THAA μg l^{-1}	%AA-C	%AA-N
27 Jul.	28℃	25 ±4.7	1.5 ±0.3	7.5 ±0.7
9:00	35℃	414 ±153	7.7 ±1.6	13.6 ±1.5
	In	12	0.6	3.6
28 Jul.	28℃	54 ±12	2.8 ±0.4	16.3 ±2.7
9:00	35℃	70 ±4.2	4.3 ±1.4	18.0 ±1.3
	In	29	1.7	9.1

Further concentrations and composition of THAA during the experiments shows that Glycine and Proline represented higher concentrations. However in higher temperature increment of Proline concentration is much higher (Fig. 3).

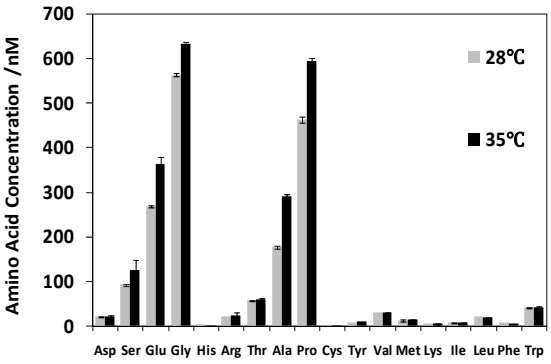

Figure 3: Variation of Concentration of the Composition of Amino Acids after two days experiments performed at 28 ^{0}C and 35^{0}C

Discussion

This experiment demonstrates new information on DON not previously reported and point's importance of linkage between coral stresses under the elevated water temperature for release of chemical substances. Results also suggest that high concentrations of nutrients and organic matters are originated by coral stress as elevation of water temperature. This indicates that organic nitrogen such as amino acids, peptides, proteins released as stress response. Considering stressed condition C: N ratio with 7 is lower and N: P ratio with 150 is higher compared with the initial samples. This observation argues that higher organic nitrogen corresponds to increase of total amino acid may be representative of peptides and proteins. Also the THAA released from coral under the heat stress supports that this possibly be peptides and or proteins. This demonstrates the considerable increment of the concentration of Proline in higher temperature could be a good representative for coral heat stress within the DON pool. However, for determination the source of organic nitrogen required further more research including microbial component. We also observed increment of bacterial abundance (Unpublished data Casareto and Suzuki), bacteria cell wall contain peptidoglycan as membrane and proteins. Future experiments will be more comprehensive after including Chemical exchange within water column, microbial component and coral biological system. We propose to include more chemical perspective in the coral biological system find the dynamics of chemical cycling by introducing the concept of chemical symbiosis within the coral animal. A simple schematic diagram in Fig. 4 explains the basic concept of chemical Symbiosis.

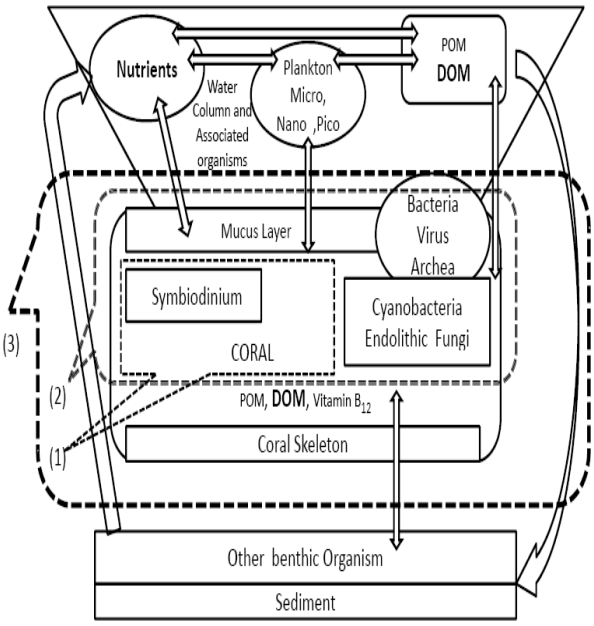

Figure 4: Proposed conceptual illustration for the chemical symbiosis with connection to detection of environment stress and physiological change in coral biological system in chemical prospective. Highlighted words reflect results to this paper. The cages represent concepts of (1) biological symbiosis, (2) holobiont and (3) chemical symbiosis.

Coral Symbiosis is well explained in coral biology and widely used in literature to show the coral animal and zoxanthellae relationship in hermatypic corals (Muscatine et al. 1984) and in microbial prospective coral system also explained as coral holobiont (Rohwer et al., 2002). However there are similarities and some differences with our proposed chemical symbiosis concept based on presentation of the idea from different perspective. Proposed concept for chemical symbiosis describes the exchange of

chemical substances such as organic material, nutrients and vitamin B_{12} (Agostini et al., unpublished) essentials for the living coral biological system and production of non living organic matrix represent as coral skeleton. Also the concept of chemical symbiosis attempt to describe environmental stress and its physiological response in the view point of exchange chemicals and this chemical signals can represents the physiological indicator either in environment or in coral organism. This is needed to reveal new insight of coral reef biogeochemical cycles.

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Exposure to nutrient-enriched sediments mitigates the negative impacts of sedimentation on coral growth in the Caribbean corals *Porites astreoides* and *Siderastrea siderea*

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Abstract. Sedimentation is a major source of disturbance to coral reefs. However, the documentation of healthy coral communities in areas with high sediment and nutrient inputs suggests that an enhanced heterotrophic environment may allow corals to thrive under marginal conditions. Here, we document the effects of sedimentation on the survivorship and growth of *Porites astreoides* and *Siderastrea siderea* to evaluate whether the higher nutrient content of enriched sediments can be utilized to offset the negative impacts of sedimentation.

P. astreoides and *S. siderea* showed high tolerance to chronic sedimentation that reduced light levels and covered corals daily for three weeks. Limited tissue losses were observed, but total mortality was not recorded. Coral growth was significantly influenced by the addition of sediments. For *P. astreoides*, control corals had the fastest growth rates, followed by corals from the enriched nutrient treatment, non-enriched sediment treatment, and the shading treatment. For *S. siderea*, growth rates were highest for control corals, followed by corals in the enriched sediments treatment, the shading treatment, and the non-enriched sediment treatment. The enhanced growth of *Porites astreoides* and *Siderastrea siderea* exposed to nutrient-enriched sediments shows that corals are able to offset the negative impacts of sedimentation by assimilating sediment nutrients.

Key words: Sedimentation, coral growth, nutrient assimilation

Introduction

The impacts of sedimentation are regarded as an increasing threat to coral reefs worldwide (Bryant et al. 1998; McCulloch et al. 2003; Fabricius 2005). Rapid population growth, changes in land-use patterns, and watershed modifications have all increased terrestrial runoff to coastal habitats (Devlin and Brodie 2005). Moreover, urban development and activities such as beach nourishment and dredging have also resulted in increased sedimentation patterns on nearshore coral reef habitats with significant detrimental effects on coral abundance, diversity, and distribution (Wilkinson 2004; Fabricius 2005).

Most sedimentation-related research on coral reefs has focused on direct and indirect stress pathways that include reduced light, abrasion, smothering, burial, toxicity (Fabricius 2005; Sanders and Baron-Szabo 2005), and associated impacts that include reduced photosynthesis (Riegl and Branch 1995; Phillips and Fabricius 2003), partial or complete tissue mortality (Rogers 1983; Nugues and Roberts 2003), reduced growth (Rice and Hunter 1992), and reduced fertilization, larval survivorship, and recruitment (Gilmour 1999; Babcock and Smith 2000). However, recent studies indicate that reef habitats exposed to sedimentation, suspended particulate matter, and nutrient inputs have access to an enhanced heterotrophic environment that may allow corals to survive and thrive under conditions often considered as marginal for coral growth and reef development (Anthony 1999, 2000, 2006; Anthony and Fabricius 2000). Increased availability of heterotrophic energy and nutrient sources in nearshore coastal habitats has been linked to higher coral growth rates (Edinger et al. 2000), increased energy storage (Anthony 2006), and increased resilience to disturbance (Grottoli et al. 2006).

The role of sediments as a potential source of nutrition for corals has been documented in studies that showed that organic matter from sediments can be transferred to the coral tissue (Rosenfeld et al. 1999), and that corals can selectively extract food particles from sediments (Mills and Sebens 1997) and assimilate the N available in sediments (Mills and Sebens 2004). In this study, we document experimentally the effects of high sedimentation on the short-term (i.e., 3 weeks) growth of two Caribbean coral species, *Porites astreoides* and

Siderastrea siderea, and evaluate whether the higher nutrient content of enriched sediments can be utilized by corals to offset the negative impacts of sedimentation.

Methods

Porites astreoides and *Siderastrea siderea* are among the most abundant species in both inshore and offshore reefs of the Florida Reef Tract (Lirman and Fong 2007) and are commonly found in areas with high sedimentation (Rogers 1990). Fragments (2-3 cm in diameter) of *P. astreoides* and *S. siderea* were extracted from a single colony of each species, thus ensuring genetic uniformity of fragments. The coral colonies were collected from a mid-channel patch reef in the northern section of the Florida Reef Tract. Mean water nutrient levels for the area where the colonies were collected were 0.6 μM DIN and 0.03 μM SRP (Lirman and Fong 2007). Nutrient levels in the seawater used in this study had higher DIN (2.1 μM) and similar levels of SRP (0.04 μM; data provided by the NOAA/AOML/South Florida Program.

The fragments were attached to the PVC platforms used to measure coral growth using underwater epoxy and, after an acclimation period of two months, were placed into the 5-L aquaria used as experimental units. Coral fragments were randomly assigned to the following treatments: (1) controls; (2) non-enriched sediments; (3) enriched sediments; and (4) shading. Replicates for each treatment were 9 fragments for *P. astreoides* and 4 fragments for *S. siderea*.

Air was supplied with air stones and seawater was added continuously at a turnover rate of approximately 6-8 times every 24 hr. A barrier constructed of plankton netting material was glued to the top of the aquaria to trap sediments as the water flowed out of the aquaria. Corals were kept in a aereated, flow-through system (as opposed to a static system) to ensure that any changes in pH, dissolved oxygen, carbonate chemistry, and water nutrients due to the addition of sediments would be transient, thus isolating the impacts of sedimentation and sediment nutrients from these other potentially confounding factors. Measurements of pH, dissolved oxygen, and salinity inside the experimental aquaria before and after the addition of sediments did not show any significant impacts of sediment addition on these parameters. However, water nutrient levels were not measured after sediment addition. While we believe that the use of a flow-through system limits the influence of nutrients transferred from the sediments to the water column as a factor in this study, the lack of nutrient readings precludes a full separation between the potential effects of sediment nutrients and elevated water nutrients.

Similarly, the seawater supplied was filtered using a set of 10 μm and 1 μm canister filters to minimize the amount of external particles available to the corals. The aquaria were placed within flow-through holding tanks for temperature control and light was supplied by compact fluorescent bulbs on a 12-hour light and dark cycle. The aquaria were cleaned and repositioned at random within the holding tanks once a week. The amount of light reduction caused by sedimentation was measured with a LiCor sensor placed inside an aquarium where levels of PAR were measured at 10 min-intervals after sediment addition.

The sediments used in this study were collected from the top layer (< 5 cm in depth) at a reef from Key Largo, Florida. Mean particle size, calculated with a Coulter Particle Size Analyzer, was 176 μm (S.D. = ± 34, n = 4 samples). The sediments were placed in two aerated 10-gal aquaria filled with seawater one week prior to the start of the experiment. Nutrients were added to one of the aquaria to create an enriched sediment treatment. NO_3 and PO_4 were added to the seawater to achieve enrichment levels of approximately 20 μM N and 2 μM P above ambient (Fong et al. 2003). At the end of the experiment, five samples were collected from the control and enriched sediments and the nutrient content of sediments was measured by the Nutrient Analytical Services Laboratory of the University of Maryland.

Each day, sediments were added to the experimental aquaria at a concentration of approximately 1000 mg DW L^{-1}. The mean sedimentation rate within aquaria was 53 mg DW cm^{-2} d^{-1} (S.D. = ± 17). These high concentrations were within the range of values reported to cause significant negative impacts on coral physiology and were similar to the sedimentation values obtained for Biscayne Bay, a coastal lagoon adjacent to the city of Miami (Rogers 1990; Lirman et al. 2003; Fabricius 2005; Weber et al. 2006). Finally, to evaluate the effects of light reduction on coral growth, corals were kept under shade-cloth that reduced available light levels by 62.5 % throughout the experiment.

Coral growth was measured using a Keyence© high accuracy CCD micrometer that measures the height profile of corals mounted on PVC platforms with micron-accuracy (Fig. 1). An aluminum pin cemented into each platform provides a standard against which coral height can be accurately measured. By measuring the height profile of each fragment repeatedly, linear extension rates can be determined as the difference in height between measurement intervals. Reported vertical linear extension rates are the average extension rate over the

entire surface of the colony. Differences in growth rates were compared among treatments using ANOVA.

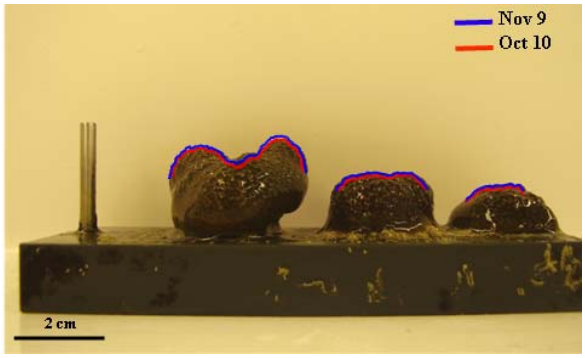

Figure 1: Photograph of the PVC platform used to measure vertical coral growth with a Keyence© high-accuracy CCD micrometer. Vertical growth along the coral contour was measured as the difference in height between intervals.

Results

Immediately after sediment addition, light levels inside aquaria were reduced to < 10 % of ambient levels but returned to pre-sedimentation levels (250 – 315 µE m^{-2} sec^{-1}) within 1.5 - 2 hr as the suspended sediments settled on the coral surface and the bottom of the aquaria. The light levels recorded after the sensor was completely covered by accumulated sediments were < 5 % of ambient levels. After addition, sediments were deposited rapidly over the coral surface and corals were covered completely within 45 min. The removal of sediments from the coral surface took up to 6 hr. Mean nutrient content was 10.3 µg g DW^{-1} (S.D. = ± 1.5) N and 2.1 µg g DW^{-1} (0.4) P for the control, non-enriched sediments, and 176.2 µg g DW^{-1} (± 62.2) N and 3.8 µg g DW^{-1} (0.4) P for the enriched sediments. The mean content of both N and P was significantly higher in the enriched sediments (t-test, $p < 0.05$). Total C (organic and inorganic) content (8 - 10 % DW) was not different between the sediment treatments (t-test, $p > 0.05$). The nutrient content of the sediments used in this experiment were similar to those values obtained from reef sediments in the Florida Reef Tract by Szmant and Forrester (1996). Enriched sediments used in this study had N values higher than those recorded for inshore reef habitats (25-140 µg g^{-1}) of the Florida Keys and P values within the range (2 -6 µg g^{-1}) observed for these habitats (Szmant 2002).

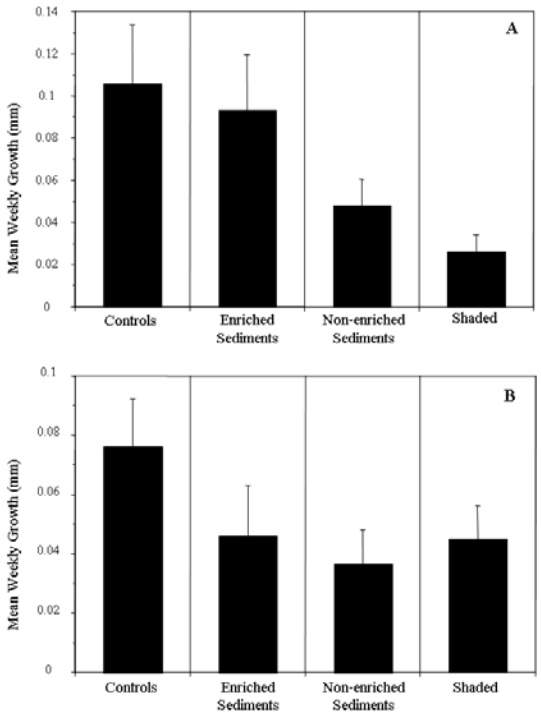

Figure 2: Growth of coral fragments of (A) *P. astreoides* and (B) *S. siderea* exposed to enriched sediments, non-enriched sediments, and shading treatments for a period of three weeks.

The growth of *P. astreoides* and *S. siderea* was significantly influenced by the addition of sediments. For *P. astreoides*, control corals had the fastest growth rates, followed by corals from the enriched nutrient treatment, non-enriched sediment treatment, and, finally, the shading treatment (Fig. 2A). No significant differences in growth rates were documented between control corals and corals exposed to enriched sediments. These two groups had growth rates significantly higher than those recorded for shaded corals and corals exposed to non-enriched sediments, which were not significantly different from each other (ANOVA, $p< 0.05$, Tukey HSD test).

For *S. siderea*, growth rates were highest for control corals, followed by corals in the enriched sediments treatment, the shading treatment, and the non-enriched sediment treatment (Fig. 2B). Significant differences were only documented between control corals and corals exposed to non-enriched sediments (ANOVA, $p < 0.05$, Tukey HSD test). Finally, some tissue losses were observed in the perimeter of the colonies, but none of the small fragments used in this study exhibited total mortality.

Discussion

Corals in marginal environments frequently encounter high levels of sedimentation and nutrients, and their

ability to maintain a positive energy balance and sustain growth under sub-optimal conditions are crucial to their persistence in these habitats (Anthony and Fabricius 2000; Lirman and Manzello 2009). The enhanced growth of *Porites astreoides* and *Siderastrea siderea* exposed to nutrient-enriched sediments shows that corals are able, at least in part, to offset the negative impacts of sedimentation by assimilating sediment nutrients. The ability of corals to assimilate nutrients and food particles contained in sediments has been previously documented by Rosenfeld et al. (1999) and Mills and Sebens (1997, 2004), but this is the first study to show a relationship between coral growth and the nutrient content of sediments. Future studies on the nutrient uptake of sediment nutrients by corals are needed to determine the nutrient pathways within the coral holobiont.

The response of corals to turbidity and sedimentation is influenced by species-specific light requirements and sediment rejection characteristics (Rogers 1983, 1990; Stafford-Smith 1993; Fabricius 2005). The present study showed a high tolerance of both *P. astreoides* and *S. siderea* to chronic high sedimentation levels that significantly reduced light levels and covered corals on a daily basis for three weeks. The ability to shed sediments and continue to grow under reduced light levels without significant tissue mortality correlates well with the high abundance of these two species throughout the Florida Reef Tract, even in areas with reduced water quality (Lirman and Fong 2007). Shading had the largest impact on the growth of *P. astreoides*, but the impact of light reduction in the absence of sediments was not significant for *S. siderea*. The green morph of *P. astreoides* used in this study is commonly found in shallow reef habitats (Gleason 1998) and may have a restricted depth distribution based on its higher light requirements.

In the Florida Reef Tract, a cross-shelf gradient in coral growth and mortality contrary to what was expected based on water quality patterns was documented by Lirman and Fong (2007). Corals from inshore habitats with higher nutrients and turbidity levels showed faster growth rates and lower rates of partial mortality compared to corals from similar habitats with lower nutrient and turbidity levels further away from shore. These findings led to the hypothesis that inshore habitats may provide an expanded heterotrophic niche for corals not available to offshore corals (Lirman and Fong 2007). This hypothesis is supported by research from Australia's GBR where corals in high-turbidity habitats are able to thrive due to particle feeding and nutrient assimilation (Anthony 1999, 2000, 2006; Anthony and Fabricius 2000). The higher growth rate of corals exposed to nutrient-enriched sediments compared to corals exposed to non-enriched sediments supports the hypothesis that the assimilation of nutrients from sediment sources may be partly responsible for the high growth rates documented for *P. astreoides* and *S. siderea* on inshore reefs where the highest levels of interstitial nutrients and N content of sediments are present (Szmant and Forrester 1996).

Finally, while enriched sediments can provide an additional source of nutrients for corals, sedimentation still remains one of the most significant sources of stress to corals worldwide. The balance between the negative impacts of sedimentation and the potential for enhanced heterotrophic sources of nutrition will depend on the frequency and intensity of sedimentation stress as well as species-specific tolerance patterns. Extensive research is still needed to fully understand the thresholds associated with sedimentation stress, but it is clear that prolonged exposure to sediments that exceed the shedding capability of corals can result in reduced growth, colony mortality, and significant modifications to coral abundance and community composition (Rogers 1990; Fabricius 2005; Dikou and van Woesik 2006). Moreover, while corals may be able to partly mitigate the cost of increased turbidity and the energy expended in clearing deposited sediments through nutrient assimilation, high organic and nutrient levels within fine sediments can cause tissue mortality through the increased activity of harmful microbial communities and the development of anoxic conditions (Weber et al. 2006).

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First evidence of coral bleaching stimulating organic matter release by reef corals

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Abstract. Corals continuously release mucoid organic exudates in order to clean their surfaces. Additionally, recent research highlighted the fact that this coral-derived organic matter acts as energy carrier and particle trap in the oligotrophic coral reef ecosystem, thus playing an important ecological role for recycling of matter and conservation of nutrients. Environmental stressors such as air exposure, high sediment loads and turbidity are known to increase the release of coral-derived organic matter. However although it is a common statement in the literature, scientific data verifying increased coral-derived organic matter release rates during temperature-induced bleaching events is lacking. This is critical as coral bleaching is the most extensive coral disease world-wide, and bleaching-induced changes in organic matter release potentially have far reaching consequences for reef functioning. In this study, a bleaching event was induced in the laboratory and release of dissolved and particulate organic carbon (DOC and POC) and nitrogen (PN) by the hermatypic coral *Acropora* spec. was quantified. Results show that during a bleaching event coral derived POC and PN release almost doubled compared to unstressed controls. This is the first experimental evidence that coral bleaching affects coral-derived organic matter release and potentially ensuing element cycles in tropical reef ecosystems.

Key Words: organic, matter, release, bleaching, stimulating

Introduction

Mucoid organic exudates continuously released by corals (Meikle et al. 1988) play an important role in heterotrophic feeding (Duerden 1906, Yonge 1930, Lewis and Price 1975, Lewis 1977, Sleigh et al. 1988, Goldberg 2002) as a defence against smothering by sediment (Schumacher 1977), desiccation (Daumas and Thomassin 1977, Krupp 1984), physical (Brown and Bythell 2005) and UVR related (Drollet et al. 1997) damage, pathogens (Ducklow and Mitchell 1979, Rublee et al. 1980, Cooney et al. 2002) or pollutants (Mitchell and Chet 1975, Neff and Anderson 1981, Bastidas and Garcia 2004). However, as mucoid organic exudates can dominate the suspended particulate matter (Johannes 1967, Marshall 1968) in reef waters, they are obviously also major components of the coral reef ecosystem's nutrient cycles. Wild et al. (2004a) suggested that these coral-derived mucoid exudates may function as energy carrier and particle trap, thereby helping to conserve essential nutrients in oligotrophic tropical coral reefs. Astonishingly, although the importance of coral-derived organic matter for reef ecosystem functioning is well documented, studies quantifying release rates in correlation to variations in the key environmental factors are rare.

During the last decades, the phenomenon of coral bleaching, i.e. the whitening of corals due to the loss of their symbiotic algae and/or pigments (Brown 1996), has become more and more evident all over the world. Mass coral bleaching events, triggered mainly by increases in water temperature, have affected the world's coral reefs with increasing frequency and intensity since the late 1970s (Hoegh-Guldberg 2004). It is predicted that due to a continued increase in seawater surface temperature (Bijlsma et al. 1995) from the year 2030 large scale bleaching events will occur annually (Coles and Brown 2003), leaving only a very short recovery period for the affected corals.

Despite the apparent actual threat of coral bleaching for the survival of coral reefs, no data is available concerning the associated release rates of coral-derived organic matter. However, such data is indispensable in order to allow any prediction concerning nutrient and energy budgets for future environmental scenarios in coral reefs. In this laboratory study, release rates of particulate organic carbon (POC), particulate nitrogen (PN) and dissolved organic carbon (DOC) by hermatypic corals of the genus *Acropora* during a temperature induced bleaching event were investigated.

In contrast to the methods of previous studies that have investigated release rates of organic matter in

relation to varying environmental factors excluding coral bleaching (Crossland 1987, Riegl and Branch 1995, Wild et al. 2005a), this study distinguished released coral-derived organic matter (mucoid exudates and host cells) from algal (zooxanthellae)-derived organic matter.

Material and Methods

Experimental description

All experiments were conducted in August and September 2007 in the aquarium facilities of the Department Biology II of LMU München, Germany. One coral colony of the genus *Acropora* was fragmented three weeks prior to the subsequent experiment in order to allow healing and regeneration. After the fragmentation, 10 coral fragments (surface Area: 72.4 - 126.2 cm²) were fixed on ceramic tiles (4.6 x 4.6 cm) using conventional coral glue. The experimental set-up consisted of two aquaria, the resident aquarium (215 L control aquarium), in which the fragments were maintained at non-heat-stress conditions, and a 30 L aquarium (bleaching aquarium), in which the temperature could be adjusted using a thermostat (HAAKE E52, Germany). The temperature in the resident aquarium was monitored by an ONSET underwater temperature logger revealing a temperature range between 25.6 °C and 29.3 °C in diurnal cycles. At the beginning of the incubation experiments, the coral fragments were placed in ten 1000 ml beakers filled with ca. 900 ml of filtered seawater (0.2 µm pore size) from the control aquarium. Manual transference into the beakers resulted in an expose to air of less than two seconds. Five beakers, each with one submersed colony (C1-C5), were placed in the control aquarium, thereby being exposed to the same temperature conditions as prior to the start of the experiment. The submersed fragments in the remaining five beakers were placed in the bleaching aquarium and acted as bleaching samples (B1-B5).

Initial water temperature for the bleaching samples was adjusted to 27 °C and kept at that temperature for 24 h, which complied with two incubation periods (one incubation period = 12 h). Introduction of compressed air ensured sufficient air supply and water circulation. After 12 h incubation, all coral colonies (B1-B5, C1-C5) were transferred to additional 1000 ml beakers filled with ca. 900 ml of freshly filtered seawater (0.2 µm). The incubation water of the precedent incubation period (IP) was kept for further processing as described below. This procedure was repeated every 12 h. After 24 h at 27 °C (IP 1 and 2), the temperature of the bleaching aquarium was raised every 12 h to a maximum of 32 °C at IP 7. Temperature was decreased to 29 °C and 27 °C for IP 8 and IP 9, respectively.

The occurrence of bleaching was defined as the point in time when zooxanthellae release rates of the bleaching samples were significantly higher than the release rates of the control samples. The surface areas of all coral fragments were measured as a reference parameter using the advanced geometry method described in Naumann et al. (submitted) and based on computer tomography reference as described by Laforsch et al. (2008).

Incubation water processing

The exact volume of the incubation water from all beakers was determined using a graduate 1000 ml glass cylinder with an accuracy of ± 20 ml. The incubation water was then stirred using a glass pipette and sub-samples (n = 1 for each parameter) were taken in order to determine the following parameters.

For subsequent **DOC measurements,** 5 ml of the incubation water were filtered through 0.2 µm syringe filters (FP 30/0.2 CA, Schleicher and Schell). The first 2 ml of the filtrate were discarded, but the following 3 ml were collected in precombusted brown glass bottles, which were instantly shock-frozen at -80 °C and kept frozen until analysis. For **POM quantification** (particulate organic matter), 50 ml of the incubation water were extracted and filtered by a vacuum filtration unit onto precombusted GF/F filters (Whatman, 25 mm diameter). Filters were dried for at least 48 h at 40 °C and kept dry until analysis. Another 50 ml were fixed with 2-3 drops of Lugol's solution and stored at room temperature for subsequent **enumeration of zooxanthellae** using counting towers and backlight microscopy at 400-times magnification (Axioplan, Zeiss Germany).

The remaining incubation water was fixed with formaldehyde (1 % formaldehyde end concentration) and stored in the dark at 4 °C until further treatment.

Organic matter analysis

POM analyses were conducted using an Elemental Analyzer NC 2500 for C- and N determinations (Carlo Erba, Italy). For calibration of the elemental content of the samples, two standards, Atropine ($C_{17}H_{23}NO_3$) and Cyclohexanone-2,4-dinitrophenylhydrazone ($C_{12}H_{14}N_4O_4$) were used. Obtained POC and PN values equal the total amount of released particulate organic matter (POMt). In order to obtain the released amounts of coral-derived particulate organic matter (POMc) the amount of released algal-derived organic matter (POMa) was subtracted from POMt**.** Consequently coral-derived organic matter is defined as any organic matter (mucoid exudates, host cells) released by the corals except algal cells.

For calculating the amount of released POMa, the POC and PN contents of a distinct number of

zooxanthellae was determined. Therefore a zooxanthellae suspension was produced by centrifugation (6000 g) of 400 ml incubation water from B2 after IP8. The pellet was resuspended in filtered seawater and the zooxanthellae concentration (9330 cells ml^{-1}) was determined (methodology see above). Dilution series of 0.1, 1.0, 10.0 and 50.0 ml of this solution were subsequently filtered in triplicates onto precombusted GF/F filters (Whatman). The filters were dried at 40 °C for at least 48 h before POM analysis as described above.

The released amounts of algal derived particulate organic matter (POMa) was determined by multiplying released numbers of zooxanthellae by the respective calculated carbon and nitrogen contents of a single *Symbiodinium* cell.

Bacteria abundances for 2 bleaching and 2 control samples were determined using standard DAPI coloration and fluorescence microscopy. Assuming a carbon content of 20 fg C per cell (Lee and Fuhrmann 1987), bacteria in the bleaching samples would account for 3.1 to 5.2 % of the total recorded C content. In the control samples, bacteria would account for 4.0 to 6.0 % respectively. In the light of these calculations microbial contribution was considered minor.

Unfortunately, bleaching samples B1, B4 and B5 showed necrosis after IP 7 (12 h at 32 °C). The incubation water of these fragments was therefore excluded from all further analyses unless otherwise stated.

DOC concentrations were determined by high temperature catalytic combustion (HTCO) using a Rosemount Dohrmann DC-190 total organic carbon (TOC) analyser and Potassium hydrogenphtalat as standard solution. Each sample was acidified by adding 100 µl of 20 % phosphoric acid and purged for 5 min in order to remove inorganic carbon. The DOC concentrations of each sample were measured five times. An outlier test was conducted, and the DOC concentrations of the remaining sub-samples were averaged.

Results

Induction of a bleaching event

A bleaching event was induced in the laboratory by exposing the investigated coral fragments to temperatures increased by 3 to 5 °C (Fig. 1a). Zooxanthellae enumeration revealed that from the IP 5 (30 °C) to the IP 9 (27 °C) significantly more (Table 1) zooxanthellae were released by the coral fragments incubated under elevated temperature compared to the controls (Fig. 1a).

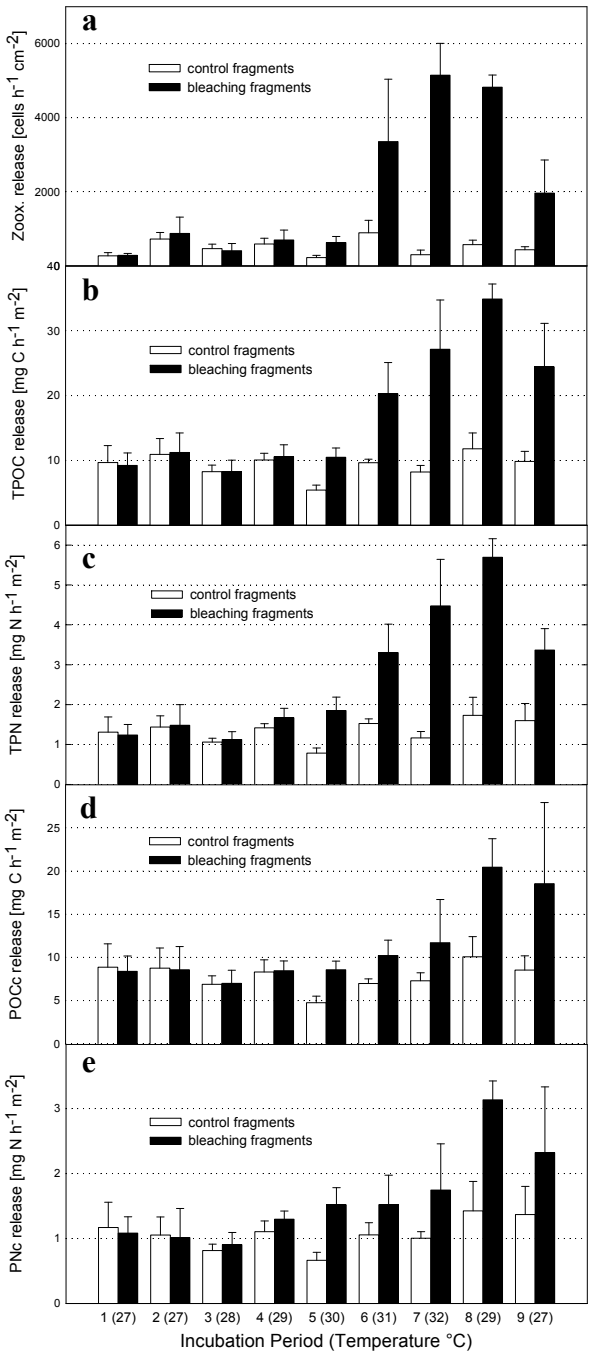

Figure 1: Summary of organic matter release rates during artificial bleaching experiment a) zooxanthellae release b) total POC release c) total PN release d) coral-derived POC release e) coral derived PN release.

Release of POCt and PNt

Throughout non-bleaching conditions there was no significant difference between the controls and the bleaching samples (Fig. 1b,c). Under bleaching conditions, from IP 5 (30 °C) until IP 9 (27 °C),

bleaching samples showed significantly (Table 1) higher POCt and PNt release rates. During IP 8 (29 °C), bleaching samples exhibited highest POCt and PNt release rates.

Regarding all bleaching samples (B1-B5), including those partially necrotic, POCt release rates were also highest during IP 8 (29 ° C) with release rates of 114 ± 75 mg C h^{-1} m^{-2} (mean ± SD, n = 5) and 14.0 ± 7.6 mg N h^{-1} m^{-2} (mean ± SD, n = 5).

Table 1: Summary of statistical analysis (independent samples t-test): given are p values for hypothesis for no differences between control fragments and bleaching fragments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Temp	Zoox.	TPOC	TPN	POCa	PNa	POCc	PNc
30 °C	***	***	***	***	***	***	***
31 °C	*	**	***	*	*	**	0.065
32 °C	***	**	***	***	***	0.079	*
29 °C	***	***	***	***	***	**	**
27 °C	**	**	**	**	**	*	0.115

Release of POMa and POMc

The released amounts of algal derived particulate organic matter (POMa) directly correlated with the zooxanthellae release rates, as POMa release was calculated by multiplying the released numbers of zooxanthellae with obtained POC and PN content of 0.3 and 0.05 ng $cell^{-1}$, respectively (linear regression of zooxanthellae numbers against respective POC content, $R^2 = 0.999$). Consequently, POCa and PNa release of the bleaching samples was significantly higher than that of the control samples whenever bleaching, as defined above, occurred (Table 1).

Algal-derived POC and PN release of the bleaching samples was lowest at IP 1 (27 °C) and highest during IP 7 (32 °C). The highest increase of algae-derived POC/PN release was found during IP 8 (32 °C), when bleaching samples released 11 to 35 times more algae-derived POC and PN than the control samples.

POMc was calculated subtracting POMa from POMt. Throughout non-bleaching conditions there was no significant difference between the treatments concerning POCc and PNc release (Fig. 1d,e). Coral-derived POC release accounted for 76 to 91 % of the total released POC and coral-derived PN for 68 to 88 % of total released PN under non-bleached conditions. Bleaching samples during IP 5 (30 °C) released significantly (Table 1) more POCc and PNc compared to the controls. During bleaching, coral derived POC release accounted for 42 to 82 % of released POCt and coral-derived PN accounted for 38 to 82 % of total released PN. Maximum POCc and PNc release could be detected during IP 8 (29 °C).

Release of DOC

No significant differences between bleaching and control samples concerning DOC concentrations could be found after any incubation period and treatment.

Discussion

Coral bleaching and organic matter release

Coral bleaching was induced at elevated temperatures (30 °C – 32 °C), but also occurred when temperature was decreased to 29 °C and 27 °C at the end of the experiment. This temporal delay may be explained by the effect of heat stress, which can lead to the breakdown of enzymatic pathways in plants and animals, resulting in metabolic or biochemical dysfunction (Cossins and Bowler 1987). Reinstalling these enzymatic pathways may take a few hours to days, depending on the damage evoked by heat stress. Thus, although temperatures were adjusted to non-bleaching conditions, the release rates were still elevated in the bleaching samples.

Besides the total release of POM, the exclusive release of particulate coral-derived organic matter was increased. This may be attributed to either increased release of mucoid exudates or increased release of coral cellular material as a consequence of the bleaching mechanism (e.g. host cell detachment). If the mechanism of bleaching, i.e. the release of zooxanthellae, was solely responsible for increased coral-derived organic matter release, the release of coral-derived organic matter should be highest when zooxanthellae release during bleaching was highest. However, coral-derived organic matter release was highest at 29 °C when zooxanthellae release had already decreased (Fig. 1a,d,e). Consequently, the mechanism of bleaching was very likely not the only factor responsible for increased coral-derived organic matter release. Therefore, increased release of mucoid exudates apparently co-occurred during bleaching.

The measured total organic matter release rates of the non-stressed control fragments are in the same range as release rates described in previous field studies (Table 2). Including coral fragments with partial necrosis, release rates were similar to those measured during air exposure (Table 2). As necrosis is one of five possible mechanisms resulting in expulsion of zooxanthellae (Gates et al. 1992), and commonly occurs during bleaching (Glynn et al. 1985), these findings underline the relevance of bleaching events for energy and nutrient cycles in the reef ecosystem. This is confirmed by the study of Wild et al. (2004b), who found that coral-derived organic matter is rapidly degraded by reef microbes, in contrast to zooxanthellae-derived organic matter, which may rather represent a loss of energy and nutrients for the reef ecosystem (Wild et al. 2005b).

Table 2: Summary of studies examining organic matter release rates by corals of the genus *Acropora*. In the present study 45 replicates are displayed, because 5 coral fragments were incubated at 9 different periods. (Note: Previous studies used old definition of mucus and did not distinguish between coral- and algal-derived organic matter).

Study site	Stress	Mucus C release (mg h^{-1} m^{-2})		Mucus N release (mg h^{-1} m^{-2})		N	Method	Reference
Heron Island	Air expos.	117 ± 79		13 ± 8		8	Container	Wild et al. 2005
Heron Island	No	10 ± 5		1.3 ± 0.8		8	Beaker	Wild et al. 2005
Heron Island	No	7 ± 3		0.8 ± 0.4		8	Beaker	Wild et al. 2005
Eilat	No	1.4 - 4.2					Perspex Chamber	Crossland 1987
Aqaba	No	1.0 - 3.0		0.1 - 0.4		5	Beaker	Naumann unpubl.
		coral-derived	algal-derived	coral-derived	algal-derived			
Laboratory	No	7.8 ± 2.1	1.5 ± 0.8	1.1 ± 0.3	0.3 ± 0.1	*45*	Beaker	This study
Laboratory	Bleach. 30°	8.6 ± 1.0	1.9 ± 0.5	1.5 ± 0.3	0.3 ± 0.1	5	Beaker	This study
Laboratory	Bleach. 31°	10.2 ± 1.8	10.1 ± 5.0	1.5 ± 0.5	1.8 ± 0.9	5	Beaker	This study
Laboratory	Bleach. 32°	11.7 ± 5.0	15.4 ± 2.6	1.7 ± 0.7	2.7 ± 0.5	2	Beaker	This study
Laboratory	Bleach. 29°	20.4 ± 3.3	14.5 ± 1.0	3.1 ± 0.3	2.6 ± 0.2	2	Beaker	This study
Laboratory	Bleach. 27°	18.5 ± 9.4	5.9 ± 2.7	2.3 ± 1.0	1.0 ± 0.5	2	Beaker	This study

This study also showed that DOC release was not influenced by coral bleaching. This is surprising as Wild et al. (2004a) demonstrated that between 56 and 80 % of coral mucus can dissolve in the surrounding seawater. However, it is very likely that a high proportion of the released DOC was re-consumed by the coral and associated bacteria (Sorokin 1973, Al-Moghrabi et al. 1993). This explanation is supported by the studies of Ferrier-Pages et al. (1998) and Naumann et al. (unpublished data), who found that it is generally difficult to detect any DOC release by corals in a closed system such as a beaker.

Furthermore, DOM polymers can spontaneously assemble to form polymer gels, thus entering the POM pool (Chin et al. 1998), which could have lead to a removal of surplus DOC from the incubation water.

Ecological implications

Increased coral-derived POM release during bleaching can probably be attributed to increased release of cellular matter and/or to increased release of mucoid exudates. Increased release of cellular matter during bleaching can be explained by the mechanism of bleaching, which may lead to loss of parts of or entire coral cells. However, the reason for increased release of mucoid exudates is harder to surmise. Up to 45 % of carbon fixed daily by the zooxanthellae can be released as organic matter by the host coral (Davies 1984, Crossland 1987, Bythell 1988, Edmunds and Davies 1989). A bleached coral is in a state of energy shortage as the algal symbionts, which are capable of providing the coral host with up to 100 % of its daily metabolic energy requirements (Muscatine et al. 1981), are lost. Thus, it is not surprising that coral bleaching can affect the release rates of organic matter.

However, there are some ecological advantages and disadvantages of up-regulation of mucoid organic matter release during coral bleaching. On one hand, energy loss via mucoid organic matter release may further reduce the ability of corals to cope with bleaching, whereas on the other hand mucoid exudates release may function for heterotrophic feeding (reviewed by Brown and Bythell 2005), which could partly compensate the missing autotrophic contribution to the coral's energy demand during bleaching. Further, increased mucoid exudates release during bleaching may also help to protect the coral against high UV radiation often associated with coral bleaching (e. g. Jokiel 1980, Fisk and Done 1985, Gleason and Wellington 1993) as UV-absorbing substances such as mycosporine-like amino acids (MAAs) have been detected in coral mucus (reviewed by Dunlap and Shick 1998).

Coral mucus may play an important role in providing various defence capabilities against pathogenic organisms (reviewed by Brown and Bythell 2005). During bleaching, corals are in a state of stress owing to energy shortage and damaged epithelia, and thus are more vulnerable to pathogens which may occur with increased abundances at elevated temperatures. Increased mucus release may be a response to decrease vulnerability and support defence against pathogens.

Recent research revealed that azoxanthellate cold water corals release POM in comparable quantities to zooxanthellate warm water corals (Wild et al. in press). This indicates that the release of mucoid exudates by corals is largely decoupled from the presence of zooxanthellae and thus represents a general response to any kind of environmental stress, including bleaching.

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Hemichordata: Enteropneusta (Acorn worm) bioturbation: Maintaining and facilitating the balance of coral reef biogeochemical cycles

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Abstract. In Bise, Okinawa, Japan, acorn worms inhabit the sandy beach, seagrass and coral/seagrass environments. As such, we assert that its bioturbation behavior aids in "biopurifying" and impoverishing the coral reef ecosystem in regards to nutrients and organic matter. Through the use of fatty acid (FA) biomarkers, C:N, and nutrient analysis, we analyzed tissues of two acorn worm species (*Ptychodera* sp. and *Schizocardium* sp.) and sediments from areas inhabited by, not inhabited by, and fecal casts of the acorn worms. Seawater samples from inhabited and not inhabited areas were analyzed for total nitrates and ammonium concentrations. FA biomarkers of acorn worm tissues indicate significant differences in diatom and plant matter consumption by the two species. In addition, acorn worms assimilate 'reactive' organic matter in the beach habitat. A significant decrease in the beach sediment overall nitrate concentration indicates acorn worm presence mitigates the release of nitrates into the water column and sediments. As the overall FA contribution and TOC contents changed little in the seagrass and coral/seagrass environments, the acorn worm demonstrates the ability to "biopurify" by subtracting "nutrient rich" organic matter, thus facilitating the maintenance of the biogeochemical balance within the coral reef ecosystem.

Key words: acorn worms, biogeochemical cycles, bioturbation, coral reef, fatty acids

Introduction

Maintaining energetic balance is essential for ecosystem function. In addition, as sediments represent the largest physiographic zone, the metabolism of benthic processes tends to dominate the coral reef ecosystem (Rasheed et al. 2002; Gattuso et al. 1998). Thus, bioturbators, which modify the sediments and directly impact benthic processes, subsequently affect the immediate, local, and ecosystem level bioenergetic cycles (Reise 1985; Tenore 1988).

In addition, as sandy beaches have been shown to act as "environmental purification systems" in coral reefs (Tsuchiya et al. 1999), and few species occupy the entire tidal gradient (Reise 1985), it is possible that deposit feeding bioturbators such as the acorn worm (Hemichordate: Enteropneusta) function as biological purifiers in the coral reef ecosystem. Thus, by assessing the role of the acorn worm, it is possible to better understand the overall impact of an organism on the biogeochemical cycles within this system.

In this study, the interactions between bioturbation and the biogeochemical cycles within the coral reef ecosystem are examined using the acorn worm.

Materials and Methods

This study was conducted in Bise, in the northern part of Okinawa Island, Japan (127° 52' 46" N, 127° 52' 43" E) from autumn 2006 to autumn 2007. The intertidal zone is characterized by beach, seagrass, and mixed coral/seagrass, habitats which are exposed during low tide. The dominant coral species is *Montipora digitata.* In addition, the dominant seagrass species is *Thalassia hemprichii.* Based on evidence of fecal cast production, acorn worms were observed at densities of up to 24 individuals m^{-2}.

Schizocardium sp. and *Ptychodera* sp. tissue samples were collected during spring and fall 2007. Sediment and water samples were collected during low tide from the beach (BE), seagrass (SG) and coral/seagrass (CS) habitats, during fall (October-November) 2006. In each habitat, the top 0.0-0.5cm of sediment was sampled with a small plastic spoon. Samples were collected from: surface sediments uninhabited (UN) by the acorn worm, surface sediments next to the fecal cast (NX, <10cm diameter from the fecal casts), and fecal casts (FC, entire fecal casts were collected). All samples were placed in plastic bags and immediately put on ice. Sediments were stored under -40°C until analysis.

Water samples, were collected from two areas within each habitat: uninhabited (UN) and inhabited (HB) by the acorn worm. 250mL of water were collected just above the sediments in the same areas. To prevent the alteration of seawater nutrients due to oxygen presence, the polyethylene bottles were opened, capped underwater and immediately put in ice and transported to the laboratory. Samples were then frozen (at -20°C) until analysis. Analysis was conducted within 24 hours of sampling.

Prior to analysis, all sediment and tissue samples were freeze-dried (FRD-51, IWAKI, Japan). Total Organic Carbon (TOC) and Total Nitrogen (TN) analysis sample preparation was carried out following the methods of Yamamuro and Kayanne (1995) for sediment samples. Analysis was conducted using a Shimadzu high sensitivity CN analyzer (Sumigraph NC-80).

Lipid and fatty acid (FA) analyses were conducted following a modified version of Bligh and Dyer (1959) as detailed by Meziane and Tsuchiya (2000). The FA biomarkers utilized for organic matter (OM) source identification are listed in Table 1.

Seawater and extracted sediment samples were analyzed for nitrate, nitrite and ammonium

Table 1. List of the FA markers used and associated OM sources identified.

Fatty acid (s)	Signature organism (OM source)
15:0 and 17:0 (*iso* and *anteiso*), 18:1ω7	Bacteria
18:2ω6, 18:3ω6, 18:3ω3	Green macroalgae
20:5ω3	Diatoms
18:4ω3, 22:6ω3	Dinoflagellates
24:0, 26:0, 28:0, 30:0, 32:0	Vascular plants

concentrations (NO_2^-+NO_3^--N and NH_4^+-N, respectively) using an automatic water analyzer (QuAAttro, Bran + Luebbe). Sediment nutrients were analyzed following the procedure outlined by Mulvaney (1996).

Data analysis was conducted using SPSS software (Standard version 11.0.1 for Windows, SPSS Inc., US). Results were considered significant if $p < 0.05$.

Results

Tissue analysis of *Ptychodera* sp. and *Schizocardium* sp. revealed significantly higher SAFAs (saturated fatty acids) and BrFAs (branched fatty acids) in *Ptychodera* sp. tissues. *Schizocardium* sp. tissue showed a significantly higher assimilation of ω3 and ω6 EFAs (essential fatty acids) (fig. 1A). Comparison of the biomarker composition of each species indicated similarities in the food resources, yet significant differences in the proportions assimilated. *Ptychodera* sp. assimilated a higher proportion of vascular plants while *Schizocardium* sp. assimilated a higher proportion of diatoms (fig. 1B).

The total organic carbon contents did not vary significantly within the beach and coral/seagrass habitat areas. In the seagrass habitat, the total organic matter (g m^{-1}) content was significantly greater in fecal casts of acorn worms than in areas uninhabited by acorn worms (ANOVA, $F = 5.3$, df = 2, 8, $p < 0.05$). Although not significant, the total amount of FAMEs tended to decrease in the fecal casts of the beach and coral/ seagrass habitats (data not shown).

In all habitats and sediment areas, saturated fatty acids (SAFAs) contributed most abundantly (between 70.1% and 45.3%) followed by monounsaturated fatty acids (MUFAs), (23-38.7%, fig. 2). Palmitic acid, 16:0 was consistently the most abundant individual FA (30.7-56.5%), followed by 16:1ω9 (18.0-30.6%).

In the beach habitat, the MUFAs (ANOVA, $F = 5.6$, df = 2, 8, $p < 0.05$), polyunsaturated fatty acids (PUFAs) (ANOVA, $F = 40.5$, df=2, 8, $p < 0.001$), and essential fatty acids (EFAs) (ANOVA, $F = 50.7$, df = 2, 8, $p < 0.001$) showed a significantly lower percent contribution to fecal cast sediments in comparison to uninhabited and next to fecal cast sediments (fig. 2A). SAFAs however, showed a significantly greater contribution (ANOVA, $F = 46.8$, df = 2, 8, $p < 0.001$).

The seagrass habitat however, only showed significantly lower contributions of PUFAs and EFAs in fecal cast sediments (fig. 2B ANOVA, $F = 11.2$, df = 2, 6, $p < 0.01$ and $F = 11.3$, df = 2, 6, $p < 0.01$, respectively). In contrast, the MUFAs, PUFAs and EFAs of fecal casts in the coral/seagrass habitat did not change significantly. Instead, the branched fatty acids (BrFAs) and long-chain fatty acids (LCFAs) showed significantly lower contributions (ANOVA, $F = 18.3$, df = 2, 6, $p < 0.01$ and $F = 6.5$, df = 2, 6, $p < 0.05$, respectively) while MUFAs showed a significantly greater contribution (ANOVA, $F = 11.1$, df = 2, 6, $p < 0.05$) in comparison to uninhabited sediments (fig. 2C).

However, in assessing the FA biomarkers (data not shown), the beach area showed a significantly lower contribution of green macroalgae and the diatom marker 20:5ω3 (eicosapentaenoic acid) in the fecal cast sediments (ANOVA, $F = 69.8$, df = 2, 8, $p < 0.001$ and $F = 56.6$, df = 2, 8, $p < 0.001$, respectively).

Although bacterial FAs did not show any differences in percent contribution to the FAMEs composition overall, the individual FA, 15:0*a* showed a significantly greater contribution in the fecal cast sediments (ANOVA, $F = 30.7$, df = 2, 8, $p < 0.01$). In contrast, the bacteria markers within fecal cast sediments of the seagrass habitat showed a

significantly lower contribution in comparison to uninhabited and next to fecal cast sediments (ANOVA, F = 37.6, df = 2, 6, p < 0.001). Green macroalgae and vascular plant markers also showed significantly lower contributions in fecal casts as compared to next to fecal cast and uninhabited areas respectively (ANOVA, F = 6.7 df = 2, 6, p < 0.05 and F = 15.4, df = 2, 6, p < 0.01).

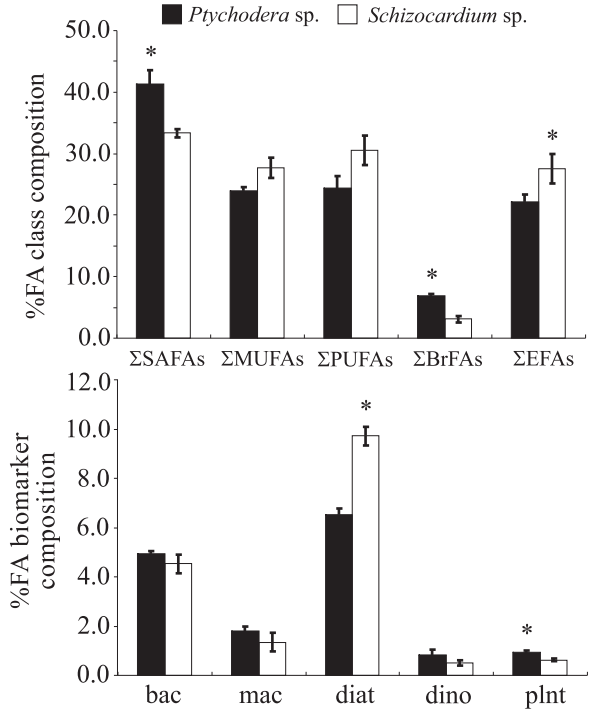

Figure 1. Fatty acid class (A) and biomarker (B) composition comparison between *Ptychodera* sp. and *Schizocardium* sp. tissues. Biomarkers are: bacteria (bac), macroalgae (mac), diatoms (diat), dinoflagellates (dino), and vascular plants (plnt). Values are averages ± SE. * indicates a significant difference, Mann-Whitney, p<0.05.

The decreases in FA markers within the coral/seagrass habitat were not consistent with either the beach or seagrass habitats. Similar to the seagrass habitat, the bacteria markers showed a significantly lower contribution in fecal cast sediments (ANOVA, F = 46.1, df = 2, 6, p < 0.001). In addition, the comparison to the other two sediment areas vascular plant markers also showed a significantly lower percent contribution to fecal cast sediments in comparison to uninhabited areas (ANOVA, F = 11.1, df = 2, 6, p < 0.05). However, although the dinoflagellate markers made up less than 1% of the overall FAMEs composition in each area, a significantly lower contribution was also seen in comparison to uninhabited sediments (ANOVA, F = 8.8, df = 2, 6, p < 0.05).

NO_2^-+NO_3^--N (hereafter referred to as NO_3^- or nitrate) and NH_4^+-N (ammonium) concentrations in seawater just above sediments uninhabited by the acorn worm and just above fecal casts of the acorn worm did not vary significantly in all habitats. However, nitrate concentrations tended to be higher than ammonium in all areas (0.05-3.05μmol l^{-1} and 0.09-0.68μmol l^{-1}, respectively). Nitrate tended to show a lower concentration in the seawater above fecal casts in all habitats whereas ammonium concentrations were slightly greater only in the coral/seagrass habitat.

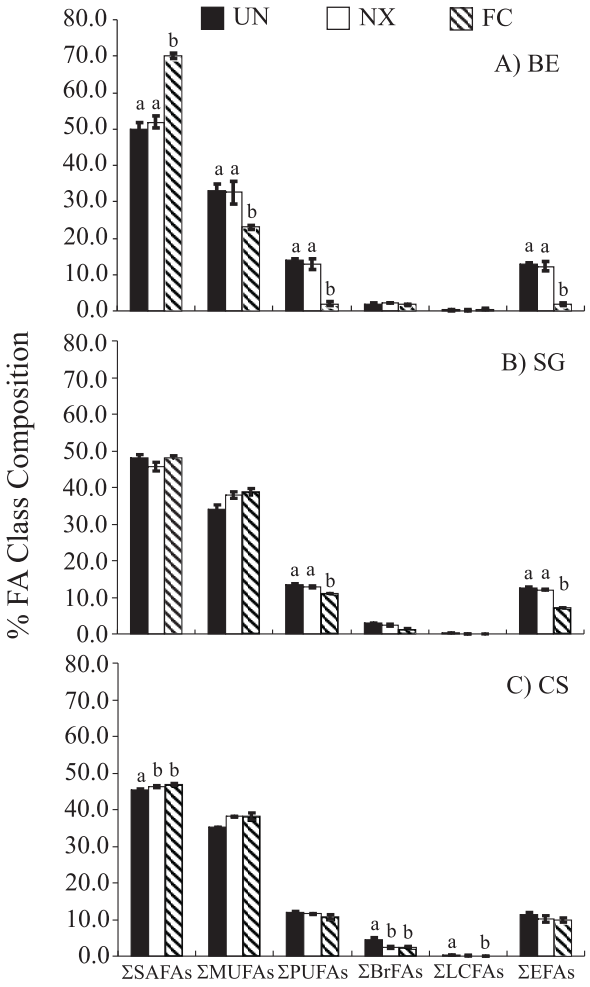

Figure 2. Habitat comparison of the percent FA class composition in the beach, seagrass, and coral/seagrass habitats between uninhabited (UN), next to fecal cast (NX) and fecal cast (FC) sediments. Values are means ± SE, lower case letters indicate significance between areas (ANOVA, p < 0.05).

In contrast, in regards to sediment nutrient content, the beach habitat showed a significantly greater concentration of ammonium than nitrate in fecal cast sediment samples (fig. 3). Here, the nitrate concentration significantly decreased in a step-wise manner from uninhabited to next to fecal cast and fecal cast sediments (fig. 3A, ANOVA, F = 279.8, df = 2, 6, p < 0.001) and the ammonium concentration

was significantly greater in fecal cast sediments (fig. 3B, ANOVA, $F = 483.6$, df = 2, 6, $p < 0.001$). In the seagrass habitat, a decreasing trend is seen in the both the nitrate and ammonium concentrations. However, the concentrations in the coral/ seagrass habitat did not vary significantly between areas.

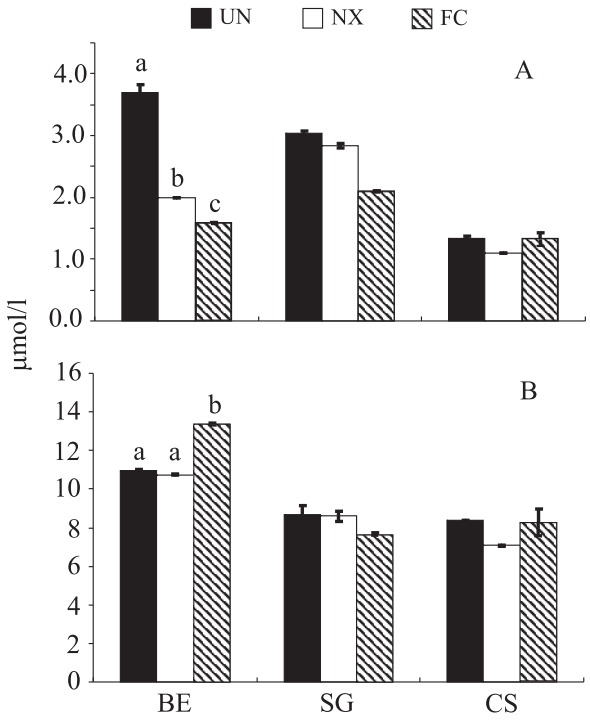

Figure 3. Area comparison of the sediment (A) nitrate and (B) ammonium concentrations in the sediments uninhabited by (UN), next the fecal casts (NX) and the fecal casts (FC) of acorn worms in the beach (BE), seagrass (SG) and coral/seagrass (CS) habitats.

The total nitrogen (TN), total organic carbon (TOC), carbon: nitrogen ratio (C:N) and total organic matter (TOM) contents in the sediment areas of the beach, seagrass and coral/seagrass did not vary significantly within all habitats.

Discussion

Tissue analysis of *Ptychodera* sp. and *Schizocardium* sp. reveals that both species primarily assimilate diatoms, followed by bacteria and macroalgae. However, significant differences in the proportions of diatoms and plants assimilated into the tissues indicates that, on a fine-scale, the impact of an acorn worm on the surrounding environment is species specific.

However, on a large-scale, the impact on the organic matter and nutrient cycles may be similar. The overall FAMEs and significant changes in OM composition exemplify the ability of the acorn worm to assimilate and biologically purify (clean) its surrounding environment, also referred to as biopurification. Similar to earthworms (Lavelle 1988), acorn worms are dependent on external biotic and abiotic parameters. This is confirmed in the inconsistent patterns of FAMEs biomarker composition (i.e. food resources) between the beach, seagrass, and coral/seagrass habitats. However, regardless of the variations due to habitat, the biopurification role of the acorn worm can still be elucidated in the overall "quality" of OM as a result of acorn worm presence. As lipids include essential fatty acids which are utilized by all organisms to maintain bodily function, the absence of EFAs in the fecal cast sediments indicates successful assimilation by the acorn worm. Consequently, the sediment processed by the acorn worm is "stripped," not "enriched" of useful FAs, , and the sediment is of less value to other OM consumers. This exemplifies biopurification.

In regards to dietary fatty acids, organisms in the marine food web have shown preferential assimilation/degradation of PUFAs, resulting in a relative increase of SAFAs (Grossi et al. 2006; Mfilinge et al. 2003). In concurrence with this, the proportional SAFA contribution was significantly higher in fecal cast sediments while PUFAs were significantly lower in the beach habitat. However, the same pattern was not seen in the seagrass and coral/seagrass habitats. Therefore, it is possible that the degradation of PUFAs is due to the preferential utilization by bacteria, rather than the direct assimilation by the acorn worm (Mfilinge et al. 2003). This is also supported by the increased contribution of MUFAs in areas near the fecal cast and fecal casts in the seagrass and coral/seagrass habitats which could instead, indicate an increase in microbial re-colonization on the fecal casts. Therefore, an indirect effect of acorn worm presence on its surrounding habitats likely includes the consequential impact on microbial utilization of nutrients in the sediments and subsequent release and/or upatake in the water column.

In addition, due to the anoxic nature of sediment, nitrate concentrations are expected to be high and ammonium low (Kogure and Wada 2005). This is exemplified by the results from each habitat. However, as the beach environment lacks the dynamics and respiration from corals, plants and other substrates, the significant decrease in nitrates and significant increase in ammonium in the fecal cast sediments clearly indicates the effects of the acorn worm presence. As such, the microbial ammonification activity in the beach habitat seems more enhanced in fecal cast sediments than in the sediment areas in the seagrass and coral/seagrass habitats.

Kogure and Wada (2005) pointed out that, although bioturbation enhances the exchange of water and

oxygen between the water column and sediment, the fecal casts of the bioturbators often remain mainly as reduced environments. Thus, if the acorn worm fecal casts contain micro-anoxic environments then the release of nitrate back into the water column would be limited by the denitrification processes occurring in reduced fecal cast environments. This indirect effect of the acorn worm presence is seen in the decreased concentrations of nitrates in the water column just above the fecal casts in the beach and seagrass habitats. Thus, perhaps a greater part of the bioturbated nitrogen in acorn worm habitats remains within the fecal casts and sediments while a lesser part is oxidized and returned to the water column.

Tenore (1988) stated that the microbial recycling of fecal material (coprophagy) allows further degredation of egested materials making re-ingestion by other organisms possible. This would result in the net increase in nitrogen and nutritional content of detritus. However, as TN concentrations remained relatively the same throughout each area, and the FA compositions showed an overall decrease in EFAs, the acorn worm fecal casts do not increase the sediment nutritional content and therefore do not encourage re-ingestion by subsequent trophic levels. Yet maintenance of the oligotrophic environment is enhanced.

The impact of bioturbators within an ecosystem is two-fold. In the case of the acorn worm, there is a direct and immediate impact on its environment based on its burrowing behavior, sediment ingestion and subsequent production of fecal casts. This "cleansing" effect is its biopurification ability. However, it also has an indirect, chronic impact on its environment based on the degradation of fecal casts over time as well as the changes in microbial dynamics as a result of acorn worm activity and presence. In addition, as acorn worms and most deposit feeders are dependent on their external, abiotic environments for resources, their subsequent influence on the environment will change depending on the season and organic matter inputs. Thus, to further understand the role of the acorn worm in the coral reef ecosystem, it is important to consider how the chronic presence of the acorn worm impacts the surrounding habitat. Studies concerning seasonal changes, acorn worm fecal cast degradation over time, microbial activity as a result of acorn worm presence, and changes in organic matter and nutrient dynamics at depth in the sediment should be conducted to further clarify the direct and indirect impacts of this bioturbator in the coral reef environment.

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New approach to study the coral symbiotic complex: Application to vitamin B12

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Abstract. Coral symbiotic complex was investigated with a new approach for sampling and measurements of vitamin B12 and bacteria abundances in the coelenteric fluid. Sampling procedure consisted in the introduction of a glass capillary using a micromanipulator into the mouths of the coral polyps, *Galaxea fascicularis,* under anesthesia and aspiration of the fluid using a vacuum pump. 0.5 to 1 ml of coelenteric fluid could be sampled by aspiration of the fluid of around 20 polyps. Flowcytometry for the measurement of bacteria abundances and radioassay with ^{57}Co labeled vitamin B12 for the measurement vitamin B12 with a minimum volume, were used. Detection limit of the radioassay was 35 pmol l^{-1} with a volume of sample of 0.4 ml. High abundances of bacteria with order of magnitude of 10^7 cell ml^{-1} and high concentrations in vitamin B12 with ranging 100 to 700 pmol l^{-1} were found in the coelenteric fluid, compared with those in surrounding water. Such approach provides a new view on the internal cycling of chemical and biological components in coral reefs. Our study it suggested that coelenteric bacteria produce vitamin B12 and that inner coral forms a semi-closed system.

Key words: Coelenteric Fluid, Radioassay, *Galaxea fascicularis*, Vitamin B12.

Introduction

Coral is described as a symbiosis among an animal, *Cnidarian*, a photosynthetic organisms, the dinoflagellate *Symbiodinium* also known as zooxanthellae, and more recently, the associated prokaryotes. The two first components of this symbiotic complex are widely accepted however the last one is now under focus. Thus coral and zooxanthellae interactions have been studied for a long time. The different methods applied for these studies included the use of carbon isotopes as tracer and separation of host cells and zooxanthellae by detaching the tissue from the skeleton and centrifugation (Muscatine 1990). These classic techniques however totally omitted the associated prokaryotes. Therefore even if associated prokaryotes were the subject of several publications most remain descriptive (Ducklow and Mitchell 1979; Rohwer et al. 2001; Rohwer et al. 2002; Kellogg 2004; Klaus et al. 2004; Bourne and Munn 2005; Koren and Rosenberg 2006; Lampert et al. 2006) and few described the mechanisms of the interactions among prokaryotes, the coral and the zooxanthellae (Herndl and Velimirov 1986; Shashar et al. 1994; Reshef et al. 2006; Rosenberg et al. 2007; Siboni et al. 2008). This lack of knowledge is partly due to the lack of methodology and to the only recent gain in interest.

Corals belong to the taxum Coelenterata and are characterized by the presence of a coelenteron which can be compare to a stomach cavity with only one aperture (i.e. the mouth). Moreover all polyps of one colony are connected together through tubes running through the coenosarc (Gladfelter 1983; Blackstone 1996). The coenosarc is composed of three tissue layers: the epidermis, the mesoglea and the endodermis. The last one is separated from the skeleton by a thin calicoblastic tissue (Goldberg 2002). Therefore coelenterons of polyps from the same colony seems to be connected together. The fluid inside the coelenteron contains, named here as "coelenteric fluid" could be shared by all polyps and is in contact with the surrounding water throughout the mouth. These observations lead to the concept that the coelenteron, the inner coral, could be considered as a semi-closed system. Furthermore zooxanthellae are known to be principally distributed in the endodermis, close to the coelenteric fluid. After all this consideration it is easy to understand the importance of studying the processes occurring in the coelenteric fluid.

In order to study coelenterate nutrition Porter (Porter 1978) described a technique to sample the coelenteric fluid of corals. On another hand Herndl et al. (Herndl et al. 1985)following Porter's method

sampled the coelenteric fluid of a giant anemone and reported for the first time the presence of bacteria living in the coelenteron. However the technique used in these two papers is not applicable directly to corals bearing small polyps as it was designed for *Montastrea cavernosa* and later applied to Giant anemone.

Here we focused our studies on the methodology required to investigate the role of the coelenteric bacteria. In order to consider as strictly as possible the processes occurring in the coelenteron we modified Porter's method to sample coelenteric fluid of small polyps corals. Also we chose vitamin B12 to investigate the linkage between the coral and the bacteria. Vitamin B12 is known to be only produced by prokaryotes. Moreover vitamin B12 is known to be required by all animals and by the majority of phytoplankton. The requirement of zooxanthellae for vitamin B12 was checked (Agostini et al., in press) and it was found that zooxanthellae were vitamin B12 dependent. For this study, measurements of vitamin B12 required the development of a new methodology with high sensitivity and requiring a low volume of sample. Determination of coelenteric bacteria abundances were done using flow-cytometry as it can be used on low volume samples. The different techniques developed and applied here are presented.

Material and Methods

Tests organisms

The coelenteric sampling technique was tested on different coral species in order to select the most suitable one. All coral colonies were taken from the reef in front of Sesoko Station, Ryukyu University, Okinawa Japan, and kept at the station in an aquarium with continuous flow of sea water. The different species tested were: *Galaxea fascicularis* (Linnaeus 1767), *Fungi fungites* (Linnaeus 1758), *Favites chinensis* (Verill 1866), and *Montastrea curta* (Dana 1864).

Coelenteric sampling

A specimen was transferred to a smaller aquarium at the time of sampling. To avoid retraction of the polyps during sampling, some corals were anesthetized using menthol (Moore, 1989). Anesthesia was done by scattering ground crystals of menthol on the surface and putting the aquarium in dark for about 45 min (depending on the species and size of the specimen and aquarium). Coral could be kept insensitive for 3 hours, until water was changed. After water change, the corals recovered quickly and therefore several anesthesia could be done in one day without any harm. Coelenteric fluid was sampled using a glass capillary mounted on a micromanipulator (Sutter Instrument model MM-33) under zoom stereomicroscope. Capillary used for the sampling was made from glass pipe and its external diameter was of less than 1 mm. The diameter should be adjusted to the polyp size. After introduction of the capillary into the mouth, the gastric content is gently sucked using a vacuum pump (max vacuum of 0.09 Bar). The fluid was collected into an HCl washed sampling tube kept in the dark during the time of sampling. Tubing connecting the capillary to the sampling tube were silicon tubes HCl washed before setup of the apparatus and between consecutive sample the tubing was washed by aspiration of MilliQ water. The volume collected by this method was of 0.5 to 1 ml after anesthesia and by sampling around 20 polyps of the same colony.

Radioassay of vitamin B_{12}

It was commonly used for determination of the level of vitamin B12 in human blood (Houts and Carney, 1981) and an attempt to use it on sea water was done by using another binder to decrease the detection limit (Sahni et al. 2001). The method used in this study was based on competitive binding radioassay using the binder and tracer from the commercial kit Simultrac-SNB (MP Biomedicals). It was optimized for seawater and required less than 0.5 ml (measurement in duplicates) of sample. Standards were done in vitamin B12 free seawater. Some samples were boiled for 30 min to check that no endogenic binder interfere with the assay. After optimization the following protocol was chosen: samples (200 µl) were incubated with 50 µl of [^{57}Co] labeled cyanocobalamin and 750 µl of Binder (purified porcine intrinsic factor bound on a solid support) for 2 hours at constant temperature and gently shaken. After centrifugation at 1000 g for 10 min, the supernatant was discarded and the pellet radioactivity was counted during 5 min using a gamma counter (Aloca, ARC-380, Tokyo Japan) set up for [^{57}Co] with a window of 100-180 KeV. Concentrations were read against standards, made with pure cyanocobalamin (Sigma Aldrich) in vitamin B12 free seawater, plotted with a logit-log scale.

Affinity of the binder in seawater was investigated by saturating aliquots of the binder with increasing amount of labeled cyanocobalamin (MP Biomedicals). Kinetics of the reaction was also investigated to determine the optimum reaction time and conditions. Incubation was done in two different conditions: at room temperature, not shaken and in a water bath at 25°C gently shaken.

Bacteria abundance determination.

In order to determine the abundances of bacteria in the coelenteric fluid flowcytometry was used (Monger and Landry, 1993). Bacteria were stained with SYBR-Green and then counted using a Beckman Coulter flow Cytometer. Picocyanobacteria were counted

using the same flowcytometer using their natural fluorescence due to main pigments, phycoerythrine and chlorophyll. Relative size of cells and their fluorescence allowed distinguishing populations of both *Prochlorococcus* and *Synechococcus*.

Results

Coelenteric sampling

Not all species were successfully sampled. Following detailed results by species may help for future applications to other species and further modifications of the technique. Mainly the results depended on the anatomy, size of the polyp.

Fungi fungites: This was the biggest polyp tested. As the polyp did not retract no anesthesia was used. Mouth of the specimen tested was more then 1 cm long and few millimeters wide. After introduction of the capillary the coral opened its mouth and secreted abundant mucus. Therefore the samples collected were largely contaminated in surrounding and freshly secreted mucus.

Favites chinensis: Size of the polyps were of the centimeter order and the mouth was almost as large as the polyp. However the sampling was not successful. First this species expands its polyps only during night time and during retraction the mouth is not accessible. Furthermore it was very sensitive: a light movement and the polyps retracted, so anesthesia in expanded state was not possible.

Montastrea curta: The size of the polyps were of the cm order and the mouth was more than 1mm. The polyp was not sensitive and introduction of the capillary was possible without anesthesia. However, this species secreted a lot of mucus and aspiration was very slow due to the high viscosity.

Galaxea fascicularis: this species was finally chosen for this study. Polyps were around 1 cm large and, depending one the specimen, could be 1 cm high. The mouth was smaller than 1 mm. The polyp was calcified until the oral disk giving it rigidity. Retraction was only of the oral disk and the tentacles which then covered the mouth. To avoid this the coral was anesthetized. The anesthesia time was of 45 min and should be done in the dark to obtain a maximum of expansion. Capillary could be inserted quite deep but it was blocked by internal calcified septum. The volume of coelenteric fluid collected by this technique was of 0.5 to 1 ml, by sampling 20 to 50 polyps in a maximum of 3 hours. Depending on the vacuum used, mesenteric filaments may be aspirated. To avoid this, pressure must be decreased but the volume and speed of collection might decrease. As a rule we limited the pressure to -0.09 Bar, usually collecting at -0.05 Bar and increasing just when necessary.

Radioassay of the vitamin B_{12}

Affinity of the binder in seawater.

Total saturation was not reached with the amount of tracer added, therefore the affinity constant was calculated using the linearisation of Hanes Woolf : the ratio between bound and added labeled cyanocobalamin was plotted against the bound vitamin (Zettner, 1973). Added vitamin was measured before centrifugation and bound in the pellet after centrifugation:

$$\frac{Vit_b}{Vit_a} = Vit_a \times \frac{1}{V_m} \square \frac{K_d}{V_m} \quad (1)$$

with Vit_b being the vitamin bound (in the pellet), Vit_a the vitamin added, V_m the maximum velocity (here the saturation), K_d the dissociation constant. For calculation of the affinity constant cpm were converted to mol l^{-1} using a specific activity of 9.02 10^{17} cpm mol^{-1}. The resulting constant of dissociation was 4.41 10^{-12} mol l^{-1}, thus the affinity was of 10^{11} l mol^{-1} which allow measurement of the picomolar order (Zettner, 1973).

Radioassay detection limit, when defined as 3 times the SD of the trace binding was of 35 pmol l^{-1} which was in agreement with the affinity constant found. The optimum conditions for the incubation were determined to be 2 hours at 25 °C gently shaken.

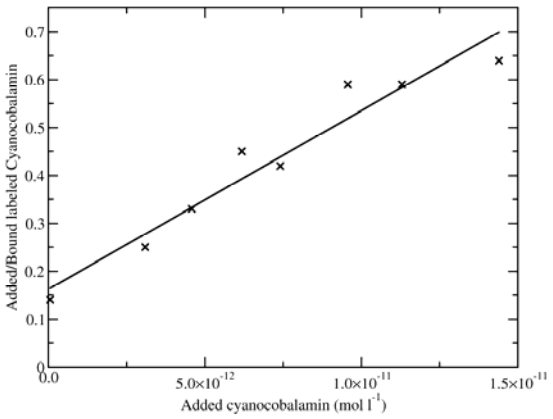

Figure 1: Linearisation of Hanes Woolf for determination of the affinity of the binder in seawater. R^2 =0.9389, slope is the inverse of the saturation and y-intercept is the ratio between Saturation and the dissociation constant

Coelenteric Fluid Analysis

Bacteria abundances

Flowcytometry was useful for the determination of coelenteric bacteria abundances. It gave reproducible results and required only 30 µl for each measurement. Abundances were always found higher in the coelenteric fluid with 7.5 x 10^6 to 4.6 x 10^7 cell ml^{-1} than those in the surrounding water: 3.54 x 10^5 cell

ml^{-1} for the aquarium water and 3.9 x 10^5 cell ml^{-1} as an average value for the natural surface water. Variations in abundances were observed but no trend, such as diurnal variation, could be observed. Picocyanobacteria (*Synechococcus* spp and *Prochlorococcus* spp) abundances were also measured one time and had a concentration similar to the aquarium water: *Synechococcus* spp : 8.9 x 10^2 cell ml^{-1} (aquarium) and 6.1 x 10^2 cell ml^{-1} (coelenteric); *Prochlorococcus* spp 4.8 x 10^2 cell ml^{-1} (aquarium) and 6.1 x 10^2 cell ml^{-1} (coelenteric).

Vitamin B_{12} concentrations

In the coelenteric fluid concentrations of vitamin B12 ranged from 108 pmol l^{-1} to 704 pmol l^{-1} . Surface water and aquarium water had no detectable level of vitamin B12 by radioassay. The difference of concentrations between coelenteric fluid and surrounding water is of 1-2 orders of magnitude (data not shown).

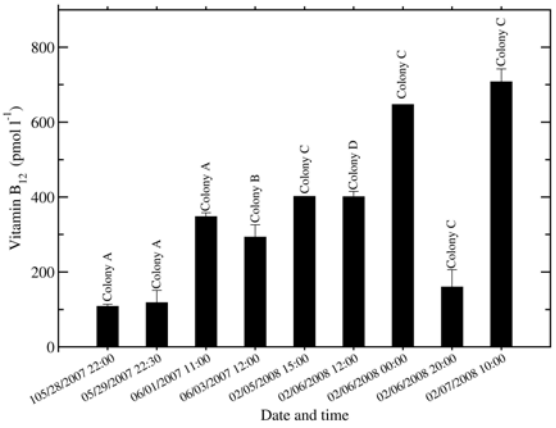

Figure 2: Coelenteric vitamin B12 found in the coelenteric fluid of Galaxea fascicularis. Colony A, B, C and D represent the 4 different colonies used. Error bars represent the standard deviation between 3 measurements.

Discussion

Radioassay allowed the measurement of vitamin B12 in the coelenteric fluid using 400 µl and the concentrations found were high and could be detected. Although the present detection limit was 30 pmol l^{-1}, by using pre concentration of the samples, lower concentrations would be detected but it will require a higher volume. Such pre concentration may be useful for the measure of vitamin B12 in the surrounding water. The affinity found of intrinsic factor, the binder used here, in saline solution (35 g l^{-1} NaCl) do not differs significantly from the one reported for blood analysis (Sahni et al. 2001). Therefore the detection limit was still comparable with the one reported for blood analysis. The affinity of intrinsic factor was reported to be maximum at pH 9 which is closed to seawater pH. Standards should be done in vitamin B12 free seawater and not in MilliQ water as affinity may be different in the two solutions leading to inaccurate concentrations. We considered that the chemical characteristics (especially pH, ionic strength and protein content) of coelenteric fluid and seawater were relatively close and therefore that differences in the affinity of the binder for vitamin B12 between standards and samples were negligible. Affinity may also change with long storage of the binder. The binder was stored in dark at 2-8°C and its stability was of around 3 months. To avoid inaccuracy, a standard curve was realized for each measurement. The original protocol provided by the maker include a step of chemical extraction. Such extraction is required for blood analysis, as in blood the vitamin B12 is bound to its transporter (Houts and Carney, 1981). Moreover the blood endogenic binder may interfere with the assay. In our case no endogenic binder were expected and in order to check their absence some samples were boiled. Such boiling would denature all proteic binder (Rothenberg, 1963). No difference between before and after boiling were noticed suggesting the absence of endogenic binder in our samples. The radioassay methods was also compared to an HPLC methods (Okbamichael and Sañudo-Wilhelmy 2004) and no significant differences for the measure of vitamin B12 concentration in seawater were found (Agostini et al., *in press*).

The sampling of coelenteric fluid of the coral *Galaxea fascicularis* was successful. No contamination from surrounding water seemed to occur as vitamin B12 concentrations and bacteria abundances differed widely. To ensure that no contamination occurred, the capillary was inserted as deep as possible and if deep insertion was not possible, aspiration was not done. Suspect samples, i.e. high volume of fluid aspirated in short time, were discarded. Vacuum pressure should be kept as low as possible to avoid aspiration of mesenteric filament and so lesion of coral tissue. However a too low pressure did not allow the collection of a sufficient volume in a reasonable time and the pressure was sometimes increase to a maximum of 0.09 Bar. Some samples required as much as 3 hours for their collection. Three hours seemed to be the maximum time for which we could keep the coral in the same water and under anesthesia. A longer time of sampling had several consequences: the coral started to show change of the color of the tissue, stability of the samples at room temperature may not be good, production or consumption by coelenteric bacteria of vitamin B12 may occur in the sampling tube. Finally a too long sampling time decrease the time resolution of the technique. In this report all time of sampling indicated the time at which the coral colony was transferred to a smaller aquarium for sampling. A shorter time of sampling may be

obtained by selecting a coral species with bigger polyps but the risk of contamination from surrounding water would increased as it happened when the coral *Fungia fungites* was tested.

Coelenteric high abundances in bacteria and high concentrations in vitamin B12 lead to 2 conclusions: (1) the coelenteric bacteria seem to produce the vitamin B12 required for the growth of the coral and symbiotic zooxanthellae. (2) the coral forms a semi-closed system enabling high concentration of essential nutrients that are scarce in the surrounding water. The concept that coral forms a semi closed system, as it was suggested by their anatomy, was confirmed by the difference between internal (inside the coelenteron) and external (in the surrounding water) vitamin B12 and bacteria abundances. Further discussion on this concept will be done elsewhere (Agostini et al*.,* in preparation).

The radioassay as described in this report provided an easy, accurate and fast way to measure vitamin B12 in a minimum of volume. Sampling of coelenteric fluid of alive coral followed by chemical and biological analysis allowed the study of the processes occurring in the coelenteron. Here we show the application of radioassay for the measure of vitamin B12 and of flowcytometry for the measure of bacteria abundances but an exhaustive description of the coelenteric fluid, biological and chemical characteristics may be required for further understanding of the relations among the different component of the coral symbiotic complex.

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Recycling-oriented aquaculture system utilizing natural ecological functions of mangrove organisms

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Abstract. Model experiments of a recycling-oriented shrimp aquaculture using natural ecological function of benthic and planktonic organisms were carried out in Samut Songkhram, the Gulf of Thailand. Water and sediment qualities in the system deteriorated during shrimp aquaculture due to the wastewater of shrimp culture. Approximately 70-75 % of nutrients were purified when water circulated and up to 2.8 kg phosphorus and 48 kg nitrogen were reduced by means of the recycling-oriented aquaculture system. Survival rate and production of shrimps were the highest in the water circulated system. Total production of the water circulated system was approximately 30 % larger than that of the closed system and, as the results, the feed conversion ratio improved about 10 %. A total of 42 species/taxa of macrobenthic organisms, mainly Annelida, Mollusca and Arthropoda, were identified and we selected species suitable for inhabit the recycling-oriented aquaculture system. The recycling-oriented aquaculture system utilizing natural ecological functions of organisms was a feasible technology for sustainable aquaculture, although further investigation should be needed.

Key words: giant tiger prawn, *Penaeus monodon*, sustainable aquaculture, macrobenthos, Thailand

Introduction

In the Gulf of Thailand, shrimp aquaculture in coastal areas has rapidly developed and expanded since the middle of the 1970's, and the production of cultured shrimps has increased drastically (DOF 2003; Barbier *et al* 2004). During the 25 years from 1975 to 2000, shrimp farming areas increased from 129 km^2 to 811 km^2 and the number of farms increased largely from 1,544 to 34,979 (DOF 2003). The excessive intensive utilization of coastal areas has resulted in the destruction of many mangrove ecosystems and water pollution affecting the culture ponds and adjacent coral reefs. Recovery of mangrove ecosystems is of utmost importance for restoring the productivity of the coastal aquaculture and fisheries (Hogarth 1999; Barbier *et al* 2004).

In order to develop a rational and sustainable utilization strategy for mangrove ecosystems, we constructed a model plant of recycling-oriented aquaculture system in which environmental loading has been reduced due to the natural ecological functions of native organisms such as macrobenthos, plankton, algae, seagrass, mangrove trees, and so on. In this study, we demonstrated one of the experiments of shrimp culture to evaluate performance of the recycling-oriented aquaculture system.

Material and Methods

Model experiments of shrimp aquaculture by means of the recycling-oriented aquaculture system were carried out in experimental ponds of Samut Songkhram Fisheries Research Station (13°26.3'N, 100°03.5'E), Kasetsart University, along the Gulf of Thailand (Fig. 1). The aquaculture system consists of five main modules (see Fig. 4); that is, shrimp culture ponds, inlet channel, outlet channel, water treatment pond and reservoir pond. Every shrimp culture ponds were 40 m x 20 m in the upper area, 35 m x 15 m in the bottom area and 1 m water depth. The inlet and the outlet channels were about 400 m x 5 m, and the water treatment pond was 80 m x 80 m with convoluted water pathway. Area of the recycling-oriented aquaculture system from the outlet channel to the inlet channel was approximately 8,500 m^2 including the water treatment pond. Mangrove saplings, *Rhizophora mucronata* Lamarck were planted into outlet channel, water treatment pond and two shrimp culture ponds. Selected various kinds of benthic organisms (mollusks, polychaetes, algae, *etc.*) were selected and cultured depending on characteristics of every modules.

The experiments were started on February 25, 2005 with shrimp fry of 15 days of age (PL15) and cultured for 160 days until August 3, 2005 (Fig. 2). 13,000 fry

of the giant tiger prawn (*Penaeus monodon* Fabricius) were stocked in each of 3 ponds (Ponds 1, 3 and 6) with a density of approximately 20 individuals per square meter (m^2), and 6000 fry in a mangrove planted pond (Pond 4). In Pond 1 and Pond 4, the water was pumped out biweekly into the outlet channel and supplied from the inlet channel through the water treatment pond. The control of the experiment was Pond 2, where no shrimps were cultured. Pond 3 was a closed intensive aquaculture without exchange of the water.

Water temperature, salinity, dissolved oxygen (DO), hydrogen-ion concentration (pH) and turbidity were monitored by means of data logger and water quality checker. Nutrient concentration were analyzed by standard methods (Eaton *et al* 1995) using a spectrophotometer and a fluorometer. Benthic organisms were quantitatively core sampling method and line intercept transect (LIT) method.

Figure 1: Overview of recycling-oriented aquaculture system in Samut Songkhram Fisheries Research Station, Kasetsart University, Thailand.

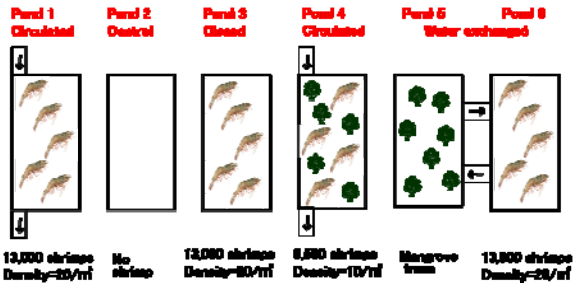

Figure 2: Experimental design of shrimp aquaculture.

Results and Discussion

Water quality during shrimp culture in Ponds 1-6 were compared in Fig. 3. Temperature fluctuated 24.3-33.9 °C in nighttime and 28.2-39.0 °C in daytime, and salinity decreased gradually because of the rainfall during rainy season. Biological oxygen demand (BOD), ammonia (NH_4) and turbidity increased gradually during shrimp culture. Water quality sometimes fluctuated because of soil erosion caused by heavy rain and phytoplankton blooming induced by strong sunshine. Water quality was stable throughout the experiment in the control pond (Pond 2), whereas, in the mangrove planted pond (Pond 4), the values fluctuated largely because of the shallow water depth (0.3 m). Dissolved oxygen (DO) decreased during the nighttime and increased in the daytime because of active photosynthesis by phytoplankton. In the daytime of fine days the rate of photosynthesis increased, and so DO and pH increased, and ammonia decreased, while the rate of photosynthesis decreased on cloudy or rainy days.

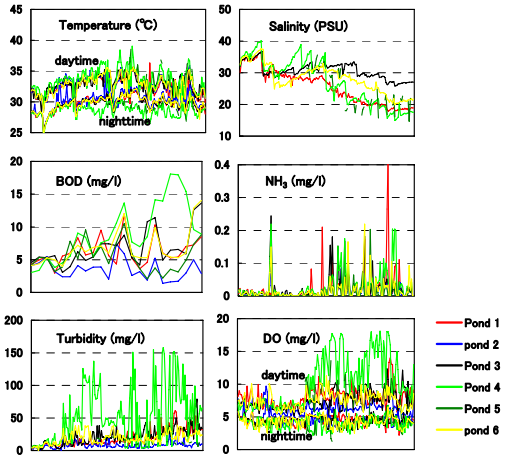

Figure 3: Water quality in experimental ponds during shrimp aquaculture.

Total nitrogen and total phosphorus were compared between before and after the shrimp culture in four modules of the recycling-oriented aquaculture system (Table 1). Before the shrimp culture, 0.001-0.010 mg/l phosphorus and 0.387-1.198 mg/l nitrogen were included in every modules. During the shrimp culture the nutrient increased gradually, and 0.150-0.483 mg/l phosphorus and 1.933-7.565 mg/l nitrogen were included when the shrimp culture terminated. Approximately 70-75 % of nutrient were purified when water circulated from the outlet channel to the inlet channel through the treatment pond; phosphorus reduced from 0.483 mg/l to 0.150 mg/l and nitrogen reduced from 7.565 mg/l to 1.933 mg/l. As the results, up to 2.8 kg phosphorus and 48 kg nitrogen were reduced by means of the recycling-oriented aquaculture system.

When water purified by means of mangrove enclosure, approximately 6.2-8.9 times of ponds was required to reduce the phosphorus (Shimoda *et al* 2005). While, in the recycling-oriented aquaculture system, approximately 5.3 times of areas are required to purify the wastewater, indicating that the system are considerably effective for promoting sustainable aquaculture.

Table 1: Nutrient in recycling-oriented aquaculture system. Total phosphorus and total nitrogen were compared among every modules before and after the shrimp culture. Unit: mg/l

	Total Phosphorus		Total Nitrogen	
	before	after	before	after
Culture pond	0.008	0.230	1.189	3.206
Outlet channel	0.010	0.483	0.610	7.565
Treatment pond	0.001	0.235	0.417	2.041
Inlet channel	0.007	0.150	0.387	1.933

A total of 42 species/taxa were identified from the recycling-oriented aquaculture system. The dominant species belong to three taxonomic categories; that is, (1) free-living nereid, *Perinereis* sp. and tube-dwelling spionid, *Polydora* sp. and some other polychaetes (Annelida), (2) gastropods such as *Cerithidea cingulata*, *Cerithium coralium*, *Sermyla riqueti*, *Stenothyra* spp. which live on the muddy or algal substrata (Mollusca), and (3) barnacles and some small arthropods such as ostracods, copepods, harpacticoids, amphipods and dipterans (Arthropoda) (Fujioka *et al* 2007). Diversity and biomass were the largest in the mangrove planted ponds and water treatment pond because of the diverse habitats associated with the mangrove trees. Whereas, they were smallest in the shrimp culture ponds because the cultured shrimps predated on macrobenthic organisms as food source.

Annelida, Mollusca and Arthropoda were the predominant and most diverse taxa in all the macrobenthic communities in the shrimp aquaculture ponds and the adjacent mangrove ecosystems (Barbier *et al* 2004; Hayashi *et al* 2005; Fujioka *et al* 2007). Since they play important roles under the natural ecosystem; that is, (1) as food for fishery resources in themselves, (2) in the purification of wastewater and sediments, and (3) in degradation, accumulation and circulation of organic materials, we utilize these organisms to maintain and improve the environment for sustainable aquaculture systems.

The results of the shrimp culture experiment were demonstrated in Table 2. During the experimental period, growth rate of shrimps differed largely among individuals from 4.5 g to more than 30 g. Survival rate and production of shrimps were the highest in the water circulated system (Pond 1). Total production of the water circulated system (Pond 1) was approximately 30 % larger than that of the closed system (Pond 2) and, as the results, the feed conversion ratio (FCR) improved about 10 %. In water exchanged system (Pond 6), growth rate of shrimps was very small possibly due to the insufficient improvement of water quality.

Cost performance of the shrimp culture experiment was also compared in Table 2. In the mangrove planted culture system (Pond 4), the artificial feed could be reduced. The profit/cost balance was the highest in the semi-intensive culture system with mangrove trees (Pond 4), followed by the water circulated system (Pond 1) and lower in the closed system (Ponds 3 and 6). Thus, effective aquaculture was achieved in the recycling-oriented aquaculture system such as Ponds 1 and 4.

Table 2: Results of shrimp culture experiment in recycling-oriented aquaculture system.

	Pond 1 Circulated	Pond 3 Closed	Pond 4 Circulated	Pond 6 Exchanged
No. of stocked (A)	13000	13000	6000	13000
Culture density (N/m^2)	20	20	10	20
No. of harvested (B)	4023	2987	1424	3142
Survival rate (%) (B/A)	30.9	23.0	23.7	24.2
Total production (Kg) (C)	58.6	41.5	24.8	25.4
Production (Kg/m^2)	0.10	0.07	0.04	0.04
Average weight (C/B) (g)	14.6	13.9	17.4	8.1
Feed (Kg) (D)	141.3	110.1	42.4	75.5
FCR (D/C)	2.41	2.65	1.71	2.98
Profit (Kg*140bahts)	8790	6225	3720	3803
Shrimp fry (n*0.11bahts)	1430	1430	660	1430
Fuel (Gasoline, Diesel Oil)	910	910	420	910
Feed (800bahts/25kg)	4520	3522	1356	2414
Profit/Cost	1.28	1.06	1.53	0.80

A considerable amount of leftover feed, exuviae and excrement of shrimps, and organic matter derived from the activities of plankton and benthos accumulated on the bottom of the aquaculture ponds and caused anaerobic conditions into the sediments. Cultured shrimp might be stressed by toxic organic materials such as hydrogen sulfide produced during shrimp culture. Such conditions are detrimental for the cultured shrimp and causes low yield of culture production and increase the susceptibility to outbreaks of disease (Barbier *et al* 2004). Controlling and minimizing the negative environmental impact by utilizing biofiltration treatment through the use of natural organisms such as mangroves trees, mollusks, polychaetes, barnacles, seaweeds and plankton is an important practical solution to maintain sustainable shrimp aquaculture.

Reducing the environmental impacts to shrimp ponds should be part of the overall issue of improving the sustainability of shrimp aquaculture. On the basis of a series of our study, we demonstrated a recycling-oriented aquaculture system in Samut Songkhram station as shown in Fig. 4. In this aquaculture model, wastewater circulated from the shrimp culture pond, though an outlet channel, to the water treatment pond. After being purified throughout the convoluted pathway in the water treatment pond, water is recycled to the shrimp culture ponds through the inlet channel. For control of DO, pH and ammonia, the density of phytoplankton should be controlled by means of a biofilter system set beside the inlet channel because they are related to the ability of photosynthesize by phytoplankton.

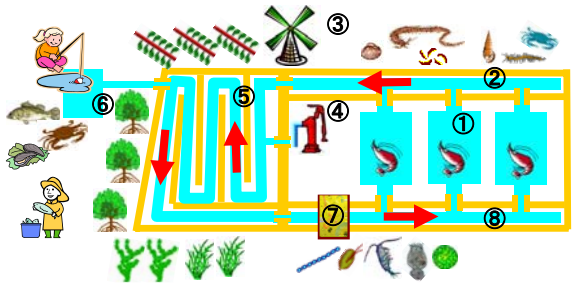

Figure 4: Recycling-oriented aquaculture model utilizing natural ecological functions of benthic and planktonic organisms. (1)shrimp culture ponds, (2) outlet channel, (3) windmill, (4) underground well, (5) water treatment pond, (6) reservoir pond, (7) biofilter, (8) inlet channel

We selected benthic organisms suitable for inhabit the recycling-oriented aquaculture system as follows: 2 mangrove (*Rhizophora mucronata* and *Avicennia marina*), 4 bivalves (*Perna viridis*, *Arcuatula arcuatula*, *Crassostrea belcheri*, *Mytilopsis adamsi*), 4 gastropods (*Cerithium coralium*, *Sermyla riqueti*, *Cerithidea cingulata*, *Stenothyra* spp.), 4 polychaetes (*Perinereis* sp., *Polydora* sp., *Mediomastus* sp., *Laonome albicingillum*), 1 barnacle (*Balanus* sp.), 1 seagrass (*Ruppia maritima*) and 5 algae (*Caulerpa lentillifera*, *Chaetomorpha* spp. *Rhizoclonium* sp., *Acanthophora spicifera* and *Solieria robusta*).

The recycling-oriented aquaculture system utilizing natural ecological functions of organisms was a feasible technology for sustainable aquaculture. For further subject, it is important to carry out the replicated experiments and improve the performance of water/sediment purification with careful screening of benthic and planktonic organisms suitable for this system.

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Activities of antioxidant enzymes (SOD and CAT) in the coral *Galaxea fascicularis* against increased hydrogen peroxide concentrations in seawater

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Abstract. The activities of two antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), were studied in a colony of *Galaxea fascicularis* corals with increased concentrations of hydrogen peroxide (H_2O_2) in seawater using an incubation chamber. Changes in enzyme activity were compared to those induced by increased seawater temperature. Three H_2O_2 concentrations (0, 0.3, and 3.0 μM) were tested at 27°C, and an elevated seawater temperature of 31°C was tested without added H_2O_2. CAT activities in both coral tissue and zooxanthellae increased with increased H_2O_2, but SOD activities remained relatively unchanged, suggesting that increased H_2O_2 in seawater affected coral cytosol but did not induce superoxide formation. In contrast, the elevated seawater temperature caused both SOD and CAT activities in coral tissue and zooxanthellae to increase. Coral bleaching was not observed at the levels of H_2O_2 tested during the 5-day exposure period. Although the long-term effects of H_2O_2 remain unknown, these results suggest that coral bleaching would likely not occur from short-term (5 days) exposure to increased H_2O_2 concentrations in seawater.

Key words: hydrogen peroxide, antioxidant enzyme, catalase, coral bleaching, *Galaxea fascicularis*

Introduction

In recent years, coral reefs have experienced extensive degradation throughout the world (Stone et al. 1999). This is the result of coral bleaching, whereby corals lose their symbiotic algal zooxanthellae or the photosynthetic pigments of the symbiotes are degraded. The causes of coral bleaching are thought to be abnormally high seawater temperatures, high levels of irradiance, strong ultraviolet (UV) radiation, changes in salinity, or a combination of these factors (Brown 1997; Fitt et al. 2001).

Environmental stress can induce increased production of reactive oxygen species (ROS), leading to significant oxidative damage to the coral-algae symbiotic system (Lesser et al. 1990; Dykens et al. 1992; Downs et al. 2002). The cellular response to the formation of oxygen radicals includes many defense mechanisms (Shick et al. 1995). For example, enzymes such as superoxide dismutase (SOD) and catalase (CAT) act in concert to inactivate superoxide radicals ($\bullet O_2^-$) and hydrogen peroxide (H_2O_2). This prevents the formation of the most reactive form of ROS, the hydroxyl radical (•OH), and subsequent cellular damage (Fridovich 1986). SOD catalyzes the dismutation of superoxide into oxygen and H_2O_2, and CAT is responsible for inactivating H_2O_2 into water and oxygen. These enzymes are responsible for detoxifying ROS, and their elevated activities indirectly indicate increased production of ROS in corals as a result of environmental stresses such as temperature, irradiance, and UV radiation (Lesser et al. 1990).

Smith et al. (2005) hypothesized that H_2O_2 may be the most important ROS associated with coral bleaching because it can act as an important signaling molecule between *Symbiodinium*, i.e., zooxanthellae, and their symbiotic host. In addition, Suggett et al. (2008) reported that the thermal tolerance of *Symbiodinium* is related to adaptive constraints associated with photosynthesis and that sensitive phylotypes are more vulnerable to H_2O_2. Furthermore, Mydlarz and Jacobs (2004) indicated that H_2O_2 production occurred as an oxidative burst in a physically injured *Symbiodinium* sp. that was isolated from *Pseudopterogorgia elisabethae*. Finally, Ross et al. (2006) reported that micromolar concentrations of H_2O_2 (>10 μM) induced cell death in the cyanobacterium *Microcystis aeruginosa* as measured by a caspase protease assay. The production of H_2O_2 was involved in the inhibition of the chlorophyll *a*-chlorophyll c_2-peridinin-protein complexes (acpPC) mRNA translation in *Symbiodinium* (Takahashi et al. 2008). Although many studies have reported the effects of in-cell H_2O_2 on zooxanthellae, few studies have examined the effects of increased H_2O_2 in the seawater surrounding a coral colony.

Here we examined the changes in activities of antioxidant enzymes (SOD and CAT) with varied concentrations of H_2O_2 in seawater and with different seawater temperatures by using a well-controlled incubation chamber. We also compared the magnitudes of changes in enzyme levels induced by H_2O_2 to those induced by high seawater temperature.

Material and Methods

Coral specimens

Colonies of *Galaxea fascicularis* were collected from a coastal region of Okinawa Island, Japan, with permission from the Okinawa prefectural government (No. 18-11). The coral colonies were immediately transferred to an outdoor tank at the University of the Ryukyus Sesoko Station of the Tropical Biosphere Research Center (TBRC). Coral branch tips (ca. 4 cm long) from three large coral colonies were cut and attached to a polycarbonate resin bolt (ca. 1 cm in diameter and 2 cm long) using glue intended for underwater use. The branch tips were kept in the outdoor tank for 3 months, after which they were moved to an indoor tank at the University of the Ryukyus Nishihara campus (ca. 60 km from TBRC) for the enzyme experiments.

Experimental design

We studied changes in coral enzyme activities using a continuous-flow complete-mixing (CFCM) experimental system. The CFCM system consists of a water tank incubation system and a flow-through system, which can continuously supply seawater while maintaining the volume of the seawater in the incubation tank during the course of the experiments. A detailed description of the CFCM is provided by Fujimura et al. (2008).

We investigated the effects of H_2O_2 on the activities of SOD and CAT in coral by controlling H_2O_2 concentrations in the supplied seawater. Various concentrations of H_2O_2 were introduced into the coral incubation tank. The H_2O_2 stock solution was prepared with 0.7 M NaCl + 2 mM $NaHCO_3$ aqueous solution to minimize decomposition of H_2O_2 and changes in salinity and carbonate concentration of the supplied seawater during the experiments. The concentration of H_2O_2 in the stock solution was determined by measuring UV absorbance at 240 nm (ε_{240} = 38.1 M^{-1} cm^{-1}; Miller and Kester 1988). Immediately before the experiments, we prepared 6 and 60 μM H_2O_2 solutions that were diluted 20-fold in the incubation tank to 0.3 and 3.0 μM, respectively. It should be noted that dilution of the filtered seawater was always kept at 20-fold throughout the experiments using the 0.7 M NaCl + 2 mM $NaHCO_3$ aqueous solution even when no H_2O_2 was added.

Seawater temperature was held constant at 27°C, and three H_2O_2 concentrations (0, 0.3, 3.0 μM) were used for 1, 3, and 5-day periods, respectively. In an additional treatment, temperature was maintained at 31°C, and no H_2O_2 (0 μM) was added. Six coral branch tips were used in each treatment. Light was provided by a metal halide lamp, and photon flux density (200 or 0 μmol m^{-2} s^{-1} during a 12:12 h light/dark cycle) was maintained throughout the experiments.

Isolation of zooxanthellae and analyses of antioxidant enzyme activities

Enzyme assays were conducted according to Levy et al. (2006) and Yakovleva et al. (2004). Tissue homogenates were prepared using the air-pick method, in which an air jet connected to a scuba regulator is used to strip the tissue from the coral skeleton into approximately 10 ml of 100 mmol l^{-1} phosphate buffer (pH=7). The volume of the homogenate was measured, and 1 ml of each subsample was used for determination of zooxanthellae density using direct counts on a Neubauer hemocytometer. A 2-ml aliquot of each homogenate sample was filtered using a GF/A filter (Whatman) and used for chlorophyll *a* (Chl. *a*) analysis. Chlorophyll was extracted from the filter overnight with acetone in the dark at 4°C. The absorbance was measured at 630 and 663 nm. We used the Jeffrey and Humphrey (1975) equation to calculate the amount of Chl. *a* extracted from the filter.

The homogenate was further centrifuged twice at 1500 × *g* for 15 min to separate the supernatant and pellets. The supernatant was used to analyze protein and enzyme activities of the host coral. The pellets containing the zooxanthellae were suspended in 2 ml of 100 mmol l^{-1} phosphate buffer, and were then dissolved by sonication for 30 min in an ice bath. A 0.05% Triton X-100 solution was added to the sonicated suspension. After incubation for 10 min, the suspension was centrifuged at 14000 × *g* for 30 min and used as the algal solution for protein and enzyme assays.

SOD activity was assayed spectrophotometrically as described by Elstner and Heupel (1976) and Oyanagui (1984). Standards for activity were prepared using bovine erythrocytic SOD (Sigma) for each set of samples. CAT activity was measured by the depletion of H_2O_2 at 240 nm (Beers and Sizer 1952). All assays were conducted at 25°C, and enzyme activity was expressed as units (U) per mg protein. Protein content was determined by the Bradford assay (Bradford 1976). Dunnett's test was used for the statistical analyses for comparing the data under various conditions against the control data (JMP 6.0.2, SAS).

Results and Discussion

Effects on SOD activity

Figure 1 shows the effects of H_2O_2 and seawater temperature on the activity of SOD in the coral tissue and in the isolated zooxanthellae. With H_2O_2 exposure, there were no significant differences in SOD activity in either coral tissue or zooxanthellae during the 5-day exposure period. Similarly, with a high seawater temperature of 31°C and without added H_2O_2, SOD activity of the host coral was relatively unchanged on day 1 and day 5, even though SOD activity of coral tissue on day 3 was significantly high. Conversely, with high seawater temperature, SOD activity in the zooxanthellae increased significantly by approximately 2-fold on day 3 and day 5 (P<0.01). Increases in antioxidant enzyme activities are indicative of increased concentrations of ROS (Lesser et al. 1990). Given that changes in SOD activity reflect changes in $\bullet O_2^-$ concentrations, the results suggested that H_2O_2 exposure did not induce $\bullet O_2^-$ formation in the coral tissue or zooxanthellae during the incubation period.

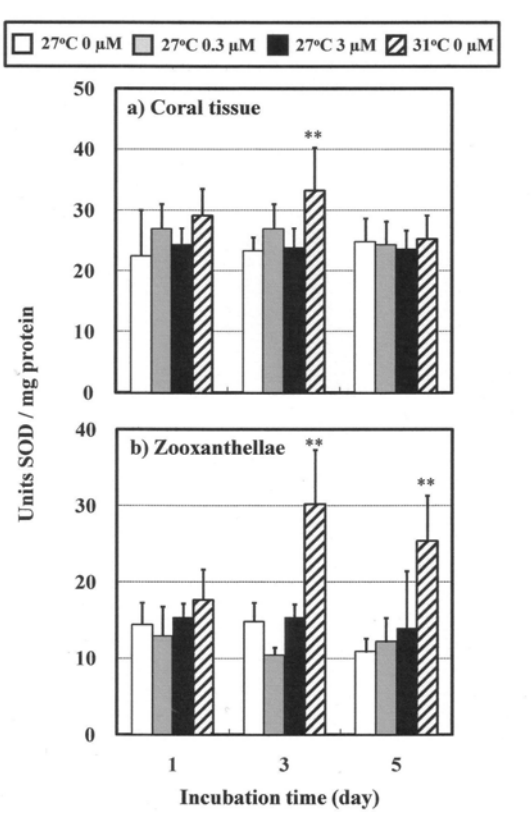

Figure 1: Effects of hydrogen peroxide and/or temperature on the activity of superoxide dismutase (SOD) in (a) coral tissue and (b) zooxanthellae. Means±S.D. (n=6).
**: P<0.01, *: P<0.05 significantly different with Dunnett's test for comparing treatments to a control.

Yakovleva et al. (2004) showed that SOD activity of coral tissue and the zooxanthellae in *Stylophora pistillata* and *Platygyra ryukyunensis* increased under high seawater temperatures, while SOD activity of the coral tissue in *P. ryukyunensis* showed little change. Dykens et al. (1992) suggested that the SOD activity in host coral changed in response to the amount of photosynthetically generated O_2 by its algal symbiotes in the sea anemone *Anthopleura elegantissima*. Our results show a greater increase in the SOD activity of zooxanthellae compared to the coral tissue, which is accordance with these studies and suggests that the zooxanthellae of the coral *Galaxea fascicularis* are more susceptible to temperature changes than the coral host is. In addition, Takahashi and Asada (1983) reported that $\bullet O_2^-$ exhibits low permeability through the cell membrane. Therefore, it is probable that the $\bullet O_2^-$ formed in the zooxanthellae did not penetrate into the coral tissues.

Effects on CAT activity

Figure 2 shows the effects of H_2O_2 and/or high water temperature on the activity of CAT in the coral tissue and in the isolated zooxanthellae. CAT activity was significantly increased by both H_2O_2 and high water temperature in coral tissue and in isolated zooxanthellae (P<0.05).

CAT activities in the host coral and in zooxanthellae increased rapidly within the first day of exposure and were similar for both increased H_2O_2 concentrations and high seawater temperature conditions. Levy et al. (2006) reported that CAT activities increased within a few minutes to several hours. Moreover, as shown in Fig. 2, CAT activities in the coral tissue increased gradually each day with either H_2O_2 exposure or high temperature conditions. It is possible that the coral tissue acclimatized day by day against increased H_2O_2 or high temperature. Yakovleva et al. (2004) also reported that the CAT activities in the coral tissue of *P. ryukyunensis* and *S. pistillata* exposed to high water temperature were higher after 12 h than after 6 h of exposure. CAT activities in zooxanthellae also increased quickly within the first day of the experiments, but CAT activities in zooxanthellae appeared to level off for the remainder of the incubation period. Yakovleva et al. (2004) also reported that CAT activities of the zooxanthellae were slightly lower after 12 h than after 6 h of exposure, which is different from the response of the host coral.

Increases in CAT activities were a result of increased H_2O_2 concentrations in the host coral and zooxanthellae, which suggests that H_2O_2 in the surrounding seawater reached the cytosol of the coral. Dykens and Shick (1982) reported that enzymatic defenses in the animal host occur in proportion to the

potential for photooxidative damage in symbiotic cnidarians. Our results showed that exposing corals to ten-fold differences in H_2O_2 concentrations did not result in significant differences in CAT activities in zooxanthellae, which suggested that zooxanthellae probably had a limit in increasing CAT activities, unless penetration of H_2O_2 into the zooxanthellae was controlled by the cell membrane. Once inside the coral cytosol, H_2O_2 should be inactivated by protective enzymes such as CAT, or could be converted into OH radicals, which could damage the photosynthesis system and can eventually lead to coral bleaching (Levy et al. 2006). Higuchi et al. (2009) showed the photosynthesis rate of the coral *G. fascicularis* was reduced by the higher concentration of H_2O_2. It was suggested that higher H_2O_2 in the coral cytosol remained unless all the H_2O_2 was neutralized by the enzymes because of the limitation of increased CAT activity.

Relationship between ROS and coral bleaching

Figure 3 shows the changes in the density of zooxanthellae, measured as the number of cells per cm^2 of the surface area of the coral colonies, during the 5-day incubation period. There were no significant differences in the density of zooxanthellae with increased concentrations of H_2O_2 during the exposures, even with an addition of 3 µM H_2O_2 (Fig. 3). Thus, coral bleaching was not observed with the elevated H_2O_2 exposure within the 5-day experiments. Downs et al. (2002) suggested that bleaching is a final defense of corals against oxidative stress. As shown in Fig. 2, corals (both host coral and zooxanthellae) were under oxidative stress with increased H_2O_2, but coral was not bleached. This could be because the oxidative stress was not due to the *in situ* formation of H_2O_2 in the symbiotic algae, and therefore the corals did not expel zooxanthellae. Alternatively, it is possible that the oxidative stress posed by the 3 µM H_2O_2 in seawater was within the tolerance level for the tested corals.

Figure 3 also shows the effects of increased seawater temperature on the density of the zooxanthellae. When seawater temperature was elevated from 27 to 31°C, the density of zooxanthellae decreased rapidly by approximately 50% within 1 day, and leveled off for the rest of the 4-day exposure. Significant differences in zooxanthellae density were observed between the corals kept at 27°C and those kept at 31°C during the 5-day high temperature conditions (P<0.05). The amount of Chl. *a* per surface area was also decreased by high temperature (data not shown).

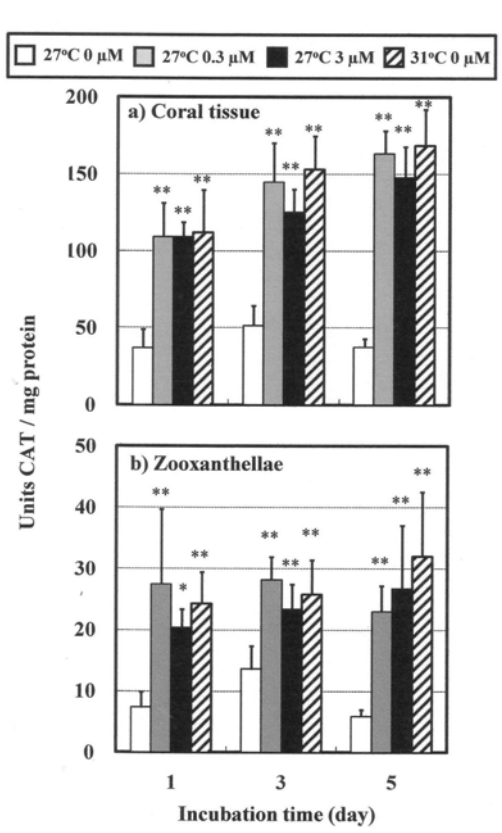

Figure 2: Effects of hydrogen peroxide and/or temperature on the activity of catalase (CAT) in (a) coral tissue and (b) zooxanthellae. Means±S.D. (n=6).
**: P<0.01, *: P<0.05 significantly different with Dunnett's test for comparing treatments to a control.

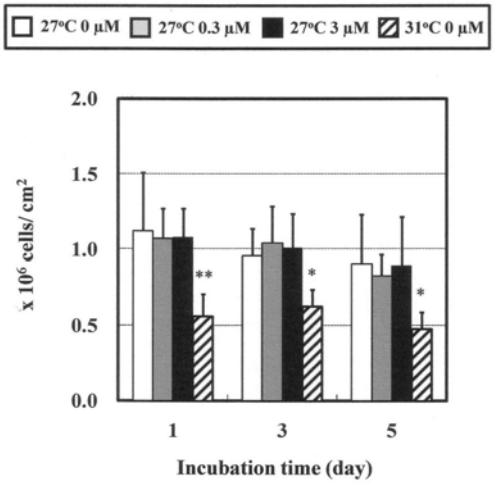

Figure 3: Effects of hydrogen peroxide and/or temperature on the density of zooxanthellae per coral surface area. Means±S.D. (n=6).
**: P<0.01, *: P<0.05 significantly different with Dunnett's test for comparing treatments to a control.

Summary

We studied the effects of increased H_2O_2 in the surrounding seawater and/or high seawater temperature on antioxidant enzyme activities of SOD and CAT in the coral *G. fascicularis*. With increased concentrations of H_2O_2, CAT activities in both coral tissue and zooxanthellae were increased, but SOD activities were relatively unaffected. In contrast, with high seawater temperature, both SOD and CAT activities in coral tissue and zooxanthellae were increased, indicating that both $\bullet O_2^-$ and H_2O_2 were formed within the cell by the increased temperature stress. CAT activities in zooxanthellae were not significantly different even with a ten-fold difference in H_2O_2 concentration. It is possible that the coral tissue and zooxanthellae reached a limit of CAT activity. Coral bleaching was not observed at the levels of H_2O_2 used for our experiments during the 5-day exposure period. Although the long-term effects of H_2O_2 remain unknown, our results suggest that coral bleaching would likely not occur as a result of short-term (5 days) exposure to increased H_2O_2 in the seawater, and that high seawater temperature had a greater impact on the enzymatic activities of the coral.

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Nitrogen dynamics in symbiotic relationships in corals

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Abstract. Symbiotic relationships in corals are found to be complex and diverse. The system supports the ecosystem in coral reefs with nitrogen cycles in usually oligotrophic environments. By introducing stable isotopes as carbon and nitrogen into seawater simultaneously, we found that uptake ratios of carbon and nitrogen fluctuate, possibly caused by variation in internal resource availability to the symbiotic system. In this study, empirical data have demonstrated imbalance of nitrogen budget that contributed in the symbiotic relationships with measurement such as nutrients in seawater and nitrogen isotope in corals. The study demonstrated mathematical model based on the findings in the incubation experiment. The model revealed that isotope method overestimate uptake rate by coral symbiosis as only uptake but not excrete rates is considered in the symbiotic relationship. Here, we propose possible method to evaluate internal and external nitrogen cycles in symbiotic system in corals. In the future, the mathematical model is going to be updated as a tool to predict symbiotic complex relationship in corals using new sampling techniques.

Key words: Nutrients, symbiosis, modeling

Introduction

Symbiotic relationships in/on corals enable corals to survive in nutrient-poor environments by exchanging metabolites and photosynthates that contain C, N, and P. Uptake and exchange of nutrients from seawater by zooxanthellae in the coral symbiotic relationships has been studied in the past (D'Elia and Cook 1988; Swanson and Hoegh-Guldberg 1998; Tanaka et al. 2001; Grover et al. 2002; Dugdale and Wilkerson 1986). Recently, the importance of internal cycling was suggested as not only based on the uptake rate but also the release rate for understanding of symbiotic mechanisms in corals (Falkowski et al. 1993).

The objectives of this study are to identify the N resource to symbiotic complex relationship between corals and zooxanthellae, to construct a preliminary mathematical model based on empirical data, and to understand an important process of internal cycling of nutrients as a critical mechanism for abundance of zooxthantellae. Until now, there is very few data on the nutrients concentrations in cnidarians (Fitt et al. 1995). In this paper, we would like to report the first data of nutrient concentration in the internal coral compared with those in seawater. We also intend to propose a new preliminary mathematical model for symbiotic complex relationships between host corals and zooxanthellae.

Material and Methods

Preparation of coral nubbins

Nubbins of 1-2 cm length of *Montipora digitata* and *Galaxea sp.* were collected from shallow water (< 1 m) at Bise, Okinawa, Japan. Nubbins were kept in a tank with running water for at least one week before experimention to minimize impacts of sampling and transportation on nubbins. All containers and instruments were washed with Extran and HCl before they were used for experiments.

Incubation experiment

A nubbin was kept in each 500 ml incubation container. Light intensity was controlled with metal halide lamps. $^{15}NO_3^-$ or $^{15}NH_4^+$, and $H^{13}CO_3^-$ were added to the seawater adjusting the final concentration to 0.07 μM, 0.05 μM, and 0.23 mM, respectively. After 6 hours of incubation period (starting between 5:00 to 7:00 under light), incubation media was sampled for nutrient measurement. Nutrients, NO_3^-, NO_2^-, PO_4^{3-}, and NH_4^+ were measured with TRAACS 2000 (BRAN+Luebbe). Nubbins were removed from the incubation container and tissues on nubbins were obtained using a WaterPik® (Johannes and Wiebe 1970). The tissue slurry was homogenized, and filtered with 47 mm GF/F filter (Whatman). The filter was acidified, dried, and analyzed for isotope ratios in carbon and nitrogen ($^{13}C/^{12}C$ and $^{15}N/^{14}N$).

Nutrient concentrations in coral nubbins

To study nutrient availability in the symbiotic complex in corals, nubbins were ground with a grinder, and 35‰ NaCl solution in Milli-Q water

(NaClaq.) was poured in the grinder. The ground coral skeleton and solution were transferred to a tube. The grinder was rinsed thoroughly with NaClaq. and combined with the solution in the tube. NaClaq. was added to the tube until the volume reached 50 ml. The tube was shaken well, and the supernatant of the tube was filtered with 0.22 μm filter unit (Millipore). Filtrate was stored as a frozen sample for nutrients measurement.

Results

Incubation experiments

The isotope ratio changes in POC and PON from nubbins were directly used to estimate uptake rate from the incubation seawater to symbiosis in corals (Dugdale and Wilkerson 1986, Grover et al. 2003). The isotope ratios of C and N in corals changed after incubation treatment. With $^{15}NO_3^-$ and $^{15}NH_4^+$ treatment, uptake rates were 6.76×10^{-8} M $cm^{-2}h^{-1}$ and 6.66×10^{-8} M $cm^{-2}h^{-1}$, respectively. C/N in taken up by coral nubbins, which was calculated from stable isotope measurement, differed among samples. Enriched with $^{15}NO_3^-$ had higher variability in C/N taken up compared with the treatment enriched with $^{15}NH_4^+$.

Nutrient concentrations in seawater sampled from incubation container decreased in scale of 10^{-2} μM over 6-hour-incubation period.

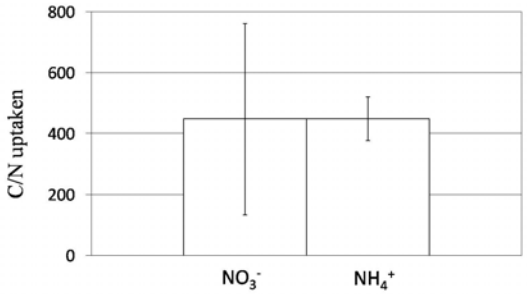

Figure1: C/N taken up by symbiosis in corals over 6 hours. $^{15}NO_3^-$ treatment had higher variation in C/N than that of $^{15}NH_4^+$ treatment. ($^{15}NO_3^-$ treatment (n=5), $^{15}NH_4^+$ treatment (n=6))

Nutrient concentrations in coral nubbins

Higher concentrations of nutrient compared with those of in seawater were detected in nubbins. Especially in nitrate and ammonium concentration in nubbins were about 100 times higher than those in seawater. The concentration varied among samples. During the treatment, nubbins excreted mucus, but the mucus influence is not subtracted from the data.

Table1: Nutrient concentration in seawater and corals. All species was higher in corals compared with those in seawater.

sample	NO_3^-	NO_2^-	NH_4^+	PO_4^{3-}
	(μM)			
Seawater	0.1	0.1	0.39-0.59	0.05-0.08
Corals	1.35-5.21	0.21-0.76	3.88-103.70	0.64-43.52

Discussion

C/N taken up by the coral symbiotic systems were variable, especially with $^{15}NO_3^-$ treatment. This implied that the behavior of C and N used by symbiotic systems in corals is not always the same. It seems that the high concentration of nutrients in nubbins supports an internal resource available besides an external resource (seawater). The high nutrient concentration in the coral symbiotic systems implied that nutrients are regenerated in a symbiotic relationship in corals (Lewis and Smith 1971; Falkowski et al. 1993; Wang and Douglas 1998). Especially the ammonium concentration is considered to be low in cells as such chemical species is detrimental to animals' health in general. Although ammonium might be excreted from corals in form of mucus in this method, the data confirmed ammonium availability in coral symbiotic systems. Whether the symbiotic relationships in corals stock nitrogen as in forms of ammonium or not is unknown. Corals may have a system to synthesize ammonium and incidentally excreting it also as mucus content. Incubation experiments with stable isotopes demonstrated that there were exchanges of nitrogen or carbon between the coral and its symbionts and the seawater even though changes of nutrient concentration in seawater appeared almost inexistant (pseudo state of exchange between surrounding sea water and interior coral). Uptake and exchange of materials from seawater to coral and symbionts has been studied in the past (Tanaka et al. 2001; Grover et al. 2002). Also, the uptake rate by symbiosis in corals has been studied (Dugdale and Wilkerson 1986; D'Elia and Cook 1988; Swanson and Hoegh-Guldberg 1998). In this study, since an internal resource was suggested, not only the uptake rate, but also the release rate was studied in symbiotic relationships in corals in order to reveal masked flow of materials in symbiotic relationships in corals (Falkowski et al. 1993).

Model construction

Here, a simplified model is built to describe symbiotic relationship in coral. In the model, to simplify the symbiotic system in corals, only zooxanthellae and

host corals were selected as symbiotic system. Uptake and release rates were given to each. As such high concentration of nutrients was found in coral nubbins, it was assumed that zooxanthellae take-up nutrients also from the host or in symbiotic systems rather than from seawater only (Fig. 2).

In the model, the host coral takes up nutrients from the surrounding water. As demonstrated in the experiment in this study, there is a possibility that host cells contain much higher nutrients than the seawater. Therefore, the uptake of nutrients by zooxanthellae was considered as uptake of nutrients from the host rather than the seawater. From acquired data and mathematical equations, the exchange rates were estimated. Concentration in seawater, host coral, and zooxanthellae were set to be C*sw*, C*host*, and C*zoox*, respectively.

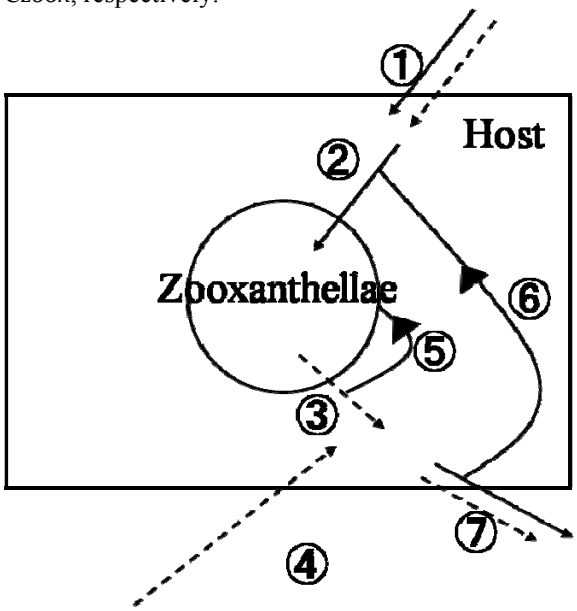

Figure 2: Suggested model of nutrient exchanges in symbiotic complex in corals. Where 1) is uptake of host from seawater, 2) is uptake by zooxanthellae from host tissue, 3) is translocation from zooxanthellae to host, 4) is host uptake of particulate matter from seawater, 5) is allocation of nutrient from zooxanthellae to zooxanthellae, 6) is allocation of nutrient from host to itself, and 7) is release of nutrients and organic matter from host to seawater. Solid arrow is inorganic, and dotted arrow is organic flow.

A mathematical model is constructed to model the nutrient interaction in symbiotic relationship in corals. The model is constructed based on data acquired from the incubation experiment, and from studies in the past. The mathematical model consists of uptake and release. Concentration changes in *s* over incubation period are rates, V*s* ($\Delta Cs/\Delta t$). The changes are integrated from time 0 to time t, then the concentration at time t becomes as the following.

$$Cs(t) = Cs(0) + \int_0^{t1} \{Vs_{uptake}(t) - Vs_{release}(t)\}dt \quad (1)$$

However, the concentration in *s* is influenced by other factors. For example, concentration in the host is influenced by uptake by zooxanthellae and degradation of particulate matter. The equation for host is

$$Chost(t) = Chost(0) + \int_0^t \{Vhost_{\text{uptake}} - Vhost_{release} + Vhost_{\text{deg}} - Vzoox_{\text{uptake}}\}dt \quad (2)$$

where V*host*$_{\text{deg}}$ is degradation rate, V*zoox*$_{\text{uptake}}$ is uptake rate of zooxnatehllae from host cell. When the each rate is integrated, C*host* (t) becomes

$$Chost(t) = Chost(0) + (\alpha - \beta + \gamma - \delta)e^t \quad (3)$$

where α, β, γ, and δ are coefficients for uptake rate, release rate, and degradation rate of particulate matter of host, and uptake rate by zooxanthellae, respectively. V*host*$_{\text{uptake}}$ rate of host was estimated from incubation experiment by using isotope in this study. With this model, major uptake of NH_4^+ from seawater was assumed to be made by host corals (Rees 1987; Miller and Yellowlees 1989). $^{15}NH_4^+$, V*host*$_{\text{uptake}}$ was estimated to be 10.9×10^{-9} mol/cm^2/h after applying the nutrient concentration dependant model, Michaelis-Menten saturation kinetics (D'Elia et al. 1983). Release rate (V*host*$_{\text{release}}$) can be estimated from NH_4^+ changes in seawater and V*host*$_{\text{uptake}}$ with assumption that V*zoox*$_{\text{release}}$ does not directly impact on NH_4^+ concentration in seawater as described in Figure 2.

$$\frac{\Delta[NH_4^+]seawater \times volume}{area \times \Delta t} = Vhost_{uptake} - Vhost_{release} \quad (4)$$

where $\Delta[NH_4^+]_{seawater}$ is NH_4^+ concentration in seawater, *volume* is volume of media used for incubation experiment, area is coral surface area, Δt is incubation time. V*host*$_{\text{release}}$ is estimated to be 11.32×10^{-9} mol/cm^2/h. Here, as uptake and release rates of host are close, isotope method could overestimate uptake rate (Shiroma et al. 2007). From equation (3), by measuring NH_4^+ concentration in host and V*zoox*$_{\text{uptake}}$ *in vivo* (Falkowski et al. 1993), V*host*$_{\text{deg}}$, can be estimated. This first step in mathematical model is useful to understand symbiotic relationship between host coral and zooxanthellae.

The study is needed to scale down the temporal as well as spatial scale to look into complex symbiotic relationship in host corals. Uptake rate for each components needs to be tracked as nutrient assimilation in dark and light are changing (Rees 1987) implying temporal changes in uptake rate. Regeneration of resource in symbiotic complex in

coral is not known in details yet (Falkowsli et al. 1993). Therefore, precise measurement of each component and mathematical model give the allocation rate of nutrient in corals (Swanson and Hoegh-Guldberg 1998). This study limited the symbiotic system by focusing on host coral and zooxanthellae; however, interaction with algae or microbes in symbiotic relationships has been studied today (Knowlton and Rohwer 2003). Empirical data focusing on this scale are needed to develop mathematical model. In addition to degradation of organic matter, the possibility of nitrogen fixation could add some more flow in Fig. 2 (Lesser et al. 2007). Laboratory experiments as well as monitoring *in situ* are required to develop the present model further. Incubation experiment demonstrated that both uptake and release rates need to be measured to estimate nutrient exchange rates in symbiotic relationship in corals. Higher concentrations of nutrients were possibly from coral skeletons, microbes, or symbiotic systems in corals such as mesoglea, stomach, and specific tissues (gastrodermis, ectoderm, or endoderm). New sampling methods will be applied (Agostini et al. 2007) in the future to determine where higher nutrients are distributed or whether the nutrients are directly available to symbiotic complex in corals.

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Long-term effects of inorganic nitrogen enrichment on the reef-building corals *Stylophora pistillata* and *Acropora* spp.

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Abstract. The increase in the nutrient level has been suggested as a major cause of localized coral reef degradation. Although the response of corals to nutrients such as ammonium or nitrate is well-documented in laboratory studies, long-term effects of continuously high inorganic nitrogen concentrations on coral physiology remained obscure. To assess the long-term effects of elevated inorganic nitrogen concentrations on the survival and growth of coral colony, we monitored the colonies of two branching reef-building corals *Stylophora pistillata* and *Acropora* spp. that had been exposed to 40 μM of NH_4^+ and 30 μM of NO_3^- over a 12 months period in flow-through aquariums. Both corals showed different responses to nutrient enrichment in survival rate, zooxanthellae density and maximum quantum yield (*Fv/Fm*). These results suggest that although both *S. pistillata* and *Acropora* spp. can adapt to relatively high inorganic nitrogen levels, faster-growing *Acropora* spp. are more susceptible to the increase in nutrient levels. The results also suggest that nutrient enrichment is not an only cause for coral reefs degradation but could results in synergistic impacts when corals are exposed to other environmental stressors.

Key words: eutrophication, growth, inorganic nitrogen, reef-building coral

Introduction

Reef-building corals typically thrive in oligotrophic ocean characterized by low levels of inorganic nutrients. They have adapted to such unique environment by establishing obligate symbiosis with dinoflagellates (zooxanthellae) that can efficiently utilize light energy through photosynthesis (D'Elia and Wiebe 1990). Since nitrogen is a limiting factor for the growth of phytoplankton, symbiotic dinoflagellate *in vivo* take up dissolved inorganic nitrogen (DIN) from surrounding water as well as utilizing coral host metabolites as nutrient sources (Muscatine and Porter 1977; Muscatine et al. 1989).

This symbiotic relationship between corals and algae is easily disrupted by environmental changes which resulted in bleaching phenomena (Hoegh-Guldberg and Smith 1989). Anthropogenic nutrient enrichment impacts on reef-building corals in direct and indirect manners both of which eventually result in the degradation of coral reef ecosystems. Reduction in coral growth and calcification (Marubini and Davies 1996), negative effects on coral reproduction (Ward and Harrison 2000), increase in coral susceptibility to infectious disease and bleaching (Bruno et al. 2003) as well as alteration of benthic community structure in coral reefs (Wielgus et al. 2004) are some of the few documented results of increased nitrogenous levels in seawater.

In contrast to our consensus that reef-building corals can only thrive in low-nutrient concentration water, there are evidences that corals do grow well in relatively high-nutrient water (Atkinson et al. 1995). In addition, a 2-year, large-scale field experiment on the GBR, Elevated Nutrient on Coral Reef Experiment (ENCORE) did not find any major impact of elevated nutrient concentrations on coral reefs at the levels of nutrient loading in areas undergoing eutrophication (Koop et al. 2001). This suggests that there is a possibility of long-term accommodation to elevated nutrients levels by corals and their symbiotic algae, as pointed out by Szmant (2002).

Although effects of elevated nitrogenous levels on corals have been demonstrated in laboratories, most of these studies were short-term (2-3 months) experiments. Among them, many of these experiments aimed at examine the hypothesis of nutrient-limitation in the coral-algae symbiotic association. In this study, we aimed to investigate the effect of elevated nitrogenous level on a relatively long-term survival and physiological changes in two branching, reef-building corals.

Material and Methods

Two species of branching, reef-building corals *Stylophora pistillata* and *Acropora* spp. were used in this study. Colonies of these corals were collected from reefs in the northern Okinawa Island, Japan and

had been acclimatized for more than a year in the flow-through-outdoor tank in Sesoko Tropical Biosphere Research Center (University of the Ryukyus). In July 2007, coral samples were cut into a similar length (approximately 3 cm) and immediately attached to acrylic screws using superglue. The coral nubbins were then acclimatized in an outdoor tank with water flow and allowed to recover for 2 weeks prior to the experiment (Nakamura and Yamasaki 2005).

Twenty four samples of each coral species were selected for the experiments. Twelve samples of each species were placed in both nutrient-enriched and control flow-through tanks (12 cm wide x 53 cm length x 7.5 cm height). Water flow rate was maintained at > 2 cm s^{-1}. Two replicates tanks were set-up for each treatment. Samples were attached to a side wall of the tanks to expose the lateral sides of coral nubbins to sunlight. The nutrient-treated tanks received seawater enriched with NH_4^+ and NO_3^- to give a final concentration of 40 μM and 30 μM, respectively. The control tanks received no nutrient supplement. The concentration of NH_4^+ and NO_3^- in the control tanks were 0.40 μM and 0.80 μM, respectively.

During the period from August 2007 to August 2008, maximum quantum yield of PS II (*Fv/Fm*) were measured weekly in coral nubbins after 15 min sunset. Buoyant weights were measured at the beginning of the experiment and at the end of the experimental period. Nutrient concentrations and flow rate were checked periodically and all tanks were cleaned every week to minimize the proliferation of algae. Data loggers (StowAway, Tidbit) were deployed to record the water temperature variability over the experimental period.

At the end of the experiment, tissue was removed from corals with a WaterPik (Johannes and Wiebe 1970), homogenized and centrifuged at 15,000 x g for 5 min (10°C). The supernatant was then discarded and the remaining zooxanthellae pellets were resuspended in a 10 ml of filtered seawater. Centrifugation was repeated 3 times. Samples were then divided into subsamples for both haemocytometer counts and pigment determination. Chlorophylls were extracted with 90% acetone and kept at 4°C for 48 h in darkness prior to the spectrophotometric determination. Chlorophyll *a* was calculated using the equations described by Jeffrey and Humprey (1975). Coral surface area was estimated using the aluminum foil method (Marsh 1970). ANOVA and *t*-test were used to analyze the data and to evaluate the differences in experimental treatments.

Results

During the course of the experiment, coral nubbins were exposed to a wide temperature ranges: summer 31°C-25°C, autumn 28°C-23°C, winter 23°C-17°C, spring 24°C-19°C. Visible bleaching in *S. pistillata* in both nutrient-enriched and control tanks was observed during summer 2007 and 2008 but not in *Acropora* spp. All bleached samples recovered from bleaching in October 2007. Coral infected by filamentous algae following the bleaching event was 33% in nutrient-enriched samples. However, no filamentous algae infection was observed in the control samples.

Concentrations of NH_4^+ and NO_3^- in enriched tanks were 100-fold and ~38-fold, respectively of those in the control tanks. Differences in coral morphology were observed between the nutrient-enriched samples and the control samples. Corals exposed to elevated nutrient concentrations exhibited darker colouration, larger polyp size, and longer polyp extension period. All coral samples survived throughout the experimental period except *Acropora* spp. that was exposed to elevated nutrient concentrations. Mortality rate was 25%.

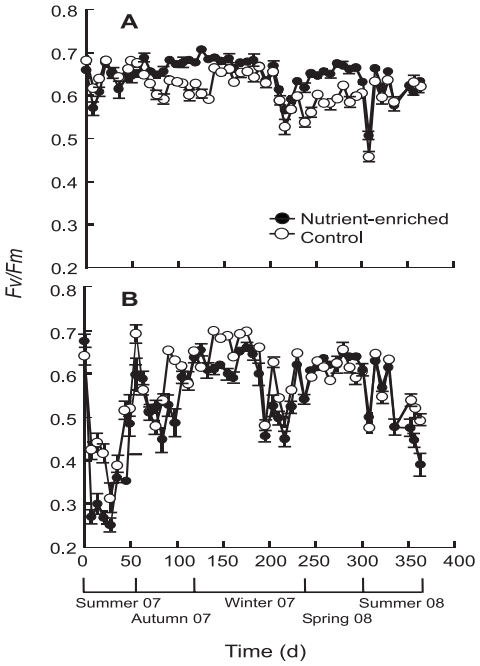

Figure 1: *Fv/Fm* changes in symbiotic algae under nutrient-enriched (filled circle) and control (open circle) conditions throughout the experimental period. (A) *Acropora* spp (B) *S. pistillata* (n = 12, SE = error bars).

Significant difference in *Fv/Fm* between nutrient-enriched samples and control samples were observed throughout the experimental period as shown in Fig 1 (ANOVA, $p<0.01$). *S. pistillata* exhibited a large fluctuation of *Fv/Fm* during the experiment period whereas *Acropora* spp. showed relatively constant

values. Larger fluctuations were observed during summer as well as winter.

Zooxanthellae density in different species of corals responded differently nutrient-enrichment (Fig 2). In the case of *S. pistillata*, significantly higher zooxanthellae density was observed for samples incubated in nutrient-enriched seawater compared to those in control (*t*-test, $p<0.001$). In contrast, in *Acropora* spp., control samples exhibited higher zooxanthellae density than the nutrient-treated samples (*t*-test, $p<0.001$).

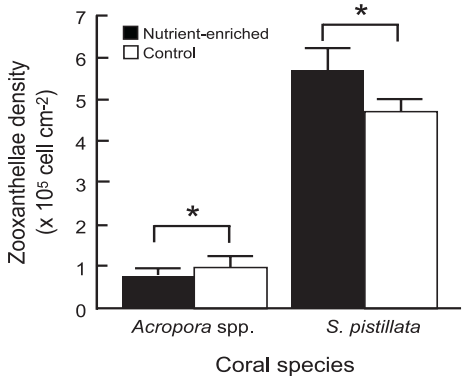

Figure 2: *Acropora* spp. and *S. pistillata*. Number of zooxanthellae per surface area of coral nubbins incubated in nutrient-enriched seawater and control seawater (n = 6, SE = error bars).

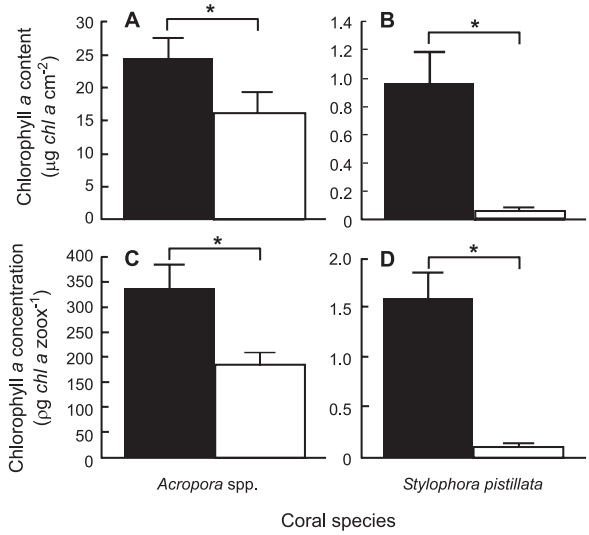

Figure 3: *Acropora* spp. and *S. pistillata*. (A and B) Chlorophyll *a* content per surface area and (C and D) Chlorophyll *a* per zooxanthella for coral nubbins incubated in nutrient-enriched seawater and control seawater (n = 6, SE = error bars).
Note: The y-axis scales are different for *Acropora* spp. and *S. pistillata*.

The most distinct effect of elevated nutrient concentration on the symbiotic algae was the increase in chlorophyll *a* content as shown in Fig 3. Chlorophyll *a* per surface area and chlorophyll *a* per algal cell were significantly greater for nutrient-treated samples compared to control samples (*t*-test, $p<0.001$). The difference between nutrient-treated samples and control samples were 10-fold higher in *S. pistillata* than in *Acropora* spp. Chlorophyll *a* content of *S. pistillata* was significantly lower compared to *Acropora* spp. (ANOVA, $p<0.001$) even though zooxanthellae density of the former was significantly higher than the later (Fig 2, ANOVA, $p<0.001$). These results are concomitant with the low *Fv/Fm* recorded in summer 2008 for *S. pistillata* as shown in Fig 1B.

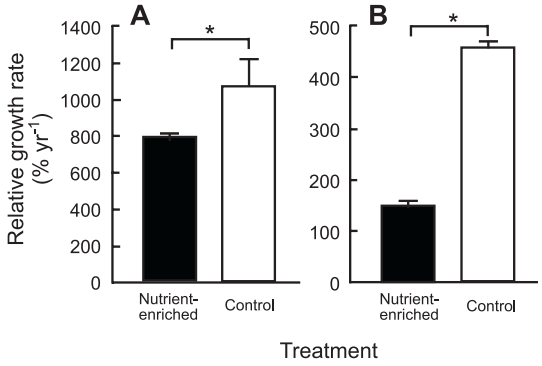

Figure 4: Differences in the relative growth rate over a 12 months period for (A) *Acropora* spp. (B) *S. pistillata* incubated in nutrient-enriched seawater (filled bar) and control seawater (open bar). (n = 6, SE = error bars).
Note: The y-axis scales are different for *Acropora* spp. and *S. pistillata*.

Significantly higher growth rate was observed in control samples than in nutrient-enriched samples (Fig 4, ANOVA, $p<0.001$). Differences in the relative growth rate (RGR) between control sample and nutrient-treated sample for *Acropora* spp. and *S. pistillata* were 1.4-fold and 3.1 fold, respectively.

Discussion

The present study has demonstrated that *Acropora* spp. and *S. pistillata* can survive in conditions of relatively high nutrient levels. However growth rates of corals were negatively affected in the nutrient-enriched treatment. In the short-term (1-2 months) experimental period, more than 50% decrease in the growth of corals exposed to 20 µM of NO_3^- or 20 µM of NH_4^+ was reported (Ferrier-Pagès et al. 2000; Marubini and Davies 1996). However, corals cultured in high-nutrient seawater at the Waikiki Aquarium, Hawaii show similar growth rates compared to those in the field (Atkinson et al. 1995). Coral reefs have formed and flourished under a wide range of natural nutrient regime (Szmant 2002). Given ample time, it is likely that coral and its symbiotic algae have the ability to adapt to relatively high nutrient levels.

Both *Acropora* spp. and *S. pistillata* showed higher chlorophyll *a* content in nutrient-treated samples although the zooxanthellae density was slightly lower in nutrient-enriched samples compared to control samples in *Acropora* spp. General response of corals towards nutrient enrichment include increase in algal density and pigmentation. This resulted in darker colouration in coral tissue (Hoegh-Guldberg and Smith 1989; Muscatine et al. 1989). The symbiotic algae in host can increase their density of thylakoids under elevated nutrient treatment (Berner and Izhaki 1994). This is likely in the case of *Acropora* spp. In contrast, *S. pistillata* might have suffered photoinhibition near the end of the experimental period which resulted in low chlorophyll *a* content.

Effect of nutrient enrichment on coral varies according to coral species. *Acropora* spp. and *S. pistillata* responded differently to nutrient enrichment. Mortality was observed in *Acropora* spp. but not in *S. pistillata*. Although the latter suffered bleaching during summer 2007 (during high SST period), it appears that they are more tolerant to elevated nitrogen level compared to the former. Elevated nutrient concentrations seem to lower the *Fv/Fm* of *S. pistillata* but may have a positive effect on *Acropora* spp. The results suggest that fast-growing *Acropora* spp. may be more susceptible to elevated nutrient levels compared to the slower-growing *S. pistillata*. This result agreed with those reported by Schlöder and D'Croz (2004).

The concentrations of NH_4^+ and NO_3^- used in this study are orders of magnitude higher than in nature as well as on polluted coral reefs. The concentration of NH_4^+ used was lower than the toxic level reported by Hoegh-Guldberg (1994). This is useful for the investigation of effects of elevated nitrogenous levels on the dynamics of coral-symbiotic association, changes in the biochemical composition of algae and long-term survival of corals.

While there are evidences that nutrient enrichment causes negative impact on coral reefs, most of the affected sites have restricted water circulation and the effects are localized (Szmant 2002). Coral nubbins were exposed to constant water flow in this study, which could have lessened the toxic effect of high inorganic nitrogen levels combined with high water temperature (Bouchard and Yamasaki 2008). Water flow reduces photodamage of algal photosynthesis as well as facilitates recovery of coral from bleaching (Nakamura et al. 2003; Nakamura et al. 2005). In general, nutrient enrichment is not the sole factor causing coral reef degradation. Corals exposed to elevated nutrient level become more susceptible to other stressors. This can lead to the decline in coral health and coral reef ecosystem.

Further studies are required in the synergistic impacts of elevated nutrient levels and other environmental stressors on the physiology of corals and coral-symbiotic association. This study suggests that effects of elevated nutrient to corals are species-dependent. Faster growing species may suffer more than slow growing one. This may be due to the differences in adaptation capability of the symbiosis system to changing environment.

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Dual-Carbon Sources Fuel the OCS Deep-Reef Community, a Stable Isotope Investigation

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Abstract. The hypothesis that phytoplankton is the sole carbon source for the OCS deep-reef community (>60 m) was tested. Trophic structure for NE Gulf of Mexico deep reefs was analyzed via carbon and nitrogen stable isotopes. Carbon signatures for 114 entities (carbon sources, sediment, fishes, and invertebrates) supported surface phytoplankton as the primary fuel for the deep reef. However, a second carbon source, the macroalga *Sargassum*, with its epiphytic macroalgal associate, *Cladophora liniformis*, was also identified. Macroalgal carbon signatures were detected among 23 consumer entities. Most notably, macroalgae contributed 45 % of total carbon to the 13C isotopic spectrum of the particulate-feeding reef-crest gorgonian *Nicella*. The discontinuous spatial distribution of some sessile deep-reef invertebrates utilizing pelagic macroalgal carbon may be trophically tied to the contagious distribution of *Sargassum* biomass along major ocean surface features.

Key words: trophic structure, Gulf of Mexico, OCS reefs, macroalgal carbon

Introduction

Trophic relationships for deep-water communities of the outer continental shelf (OCS) remain poorly studied, particularly in warm temperate and tropical oceans. The present study investigated tissue ^{13}C and ^{15}N stable isotope signatures for the fauna of deep-reef biotopes at 65-120 m on the northeastern Gulf of Mexico (NEGOM) OCS. The objective for this phase of a broader surface-to-substrate ecosystem trophic investigation was to identify carbon sources fueling the NEGOM deep-reef fauna.

Coastal and offshore ecosytems are fueled by organic carbon in the form of dissolved organic carbon (DOC) and/or particulate organic matter (POM) from photosynthesis, sometimes supplemented by chemosynthesis. Terrestrial C3 plants, emergent saltmarsh C4 plants (Thayer et al. 1978), submerged aquatic seagrasses, and benthic macroalgae may also supply carbon to nearshore ecosystems (Thayer et al. 1983). For OCS ecosystems, the only carbon source ubiquitously and continuously available is phytoplankton, classically considered the sole oceanic carbon source (Thayer et al. 1983; Fry and Sherr 1984; Dauby 1989), excluding local primary production from seafloor chemosynthesis (Kennicutt et al. 1985; Paull et al. 1985; Brooks et al. 1987). At high latitudes, sea ice algae may be an important supplementary carbon source (Hobson et al. 1995, 2002). Similarly, benthic macroalgae may contribute carbon to some coastal ecosystems (Dunton and Schell 1982; Stephenson et al. 1984; Simenstad and Wissmar 1985; Dunton and Schell 1987). However, the potential role of pelagic macroalgae as a distinct and important oceanic carbon source has generally been little investigated.

Material and Methods

Study area and sample collection. Sampling was conducted hard-bottom reef areas [ESM: Fig. 1] on the Mississippi-Alabama OCS west of DeSoto Canyon, and the West Florida OCS to the east, during three U.S. Geological Survey (USGS) cruises (2001-2003). Potential carbon sources sampled included near-surface and near-bottom plankton, pelagic macroalgae, and bottom sediment. Holoplankton and POM were sampled simultaneously using horizontal 5-min tows of nested 0.5 m diameter plankton nets, an inner 335 μm mesh net to retain holoplankton, within a 125 μm mesh net to retain POM. Near-surface nets were towed at 3-5 m depth, near-bottom nets at 5-10 m altitude. Bottom sediment was sampled in a 0.0484 m^2 box grab. Consumers sampled included benthic, benthopelagic, and epipelagic fishes and invertebrates. Samples were obtained by angling, fish traps, otter and sled trawls, tangle device, and ROV suction sampler. Macroalgae, attached epibiota, and associated fauna, were obtained in dip nets and 1.0 m diameter plankton nets.

A target of $N \geq 20$ samples per trophic entity was set, attempting to obtain sample sizes sufficient to determine statistically robust isotopic means and standard deviations (Monteiro et al. 1981; Fry and Sherr 1984). Carbon source spectrum analysis

(Jennings et al. 1997) was undertaken when sample N ≥ 20 for a given consumer entity.

Sampling at sea and laboratory preparation. For most fishes a 1-5 g sample of dorsal white muscle was excised, excluding scales, skin and bone. This tissue displays the least variance in ^{13}C and ^{15}N among all fish tissues, and is low in problematic inorganic carbonates and lipids (Pinnegar and Polunin 1999). Slow white muscle turnover rates integrate carbon source signatures over months to years (Hesslein et al. 1993; Post 2002). For invertebrates, a 1-5 g sample of soft tissue was obtained. A 2.0 ml subsample of undisturbed surface sediment was taken from each box grab sample. Outer leaf blades of *Sargassum* spp. (least enriched in ^{13}C: Stephenson et al. 1984, Ishihi et al. 2001) were scraped with a scalpel to remove encrustations, rinsed with de-ionized water (DI), and frozen. *Cladophora liniformis* samples were harvested from *Sargassum*. Plankton samples (excluding fish larvae, chaetognaths, and gelatinous macroplankton) were concentrated into a plug by vacuum filtering through a micropore filter.

Invertebrate tissue and sediment samples were each divided into two portions. One portion was treated with 3N HCl for 48 h to dissolve inorganic carbonates, DI rinsed, centrifuged, dried and plated for assay. Only $\delta^{13}C$ was determined from acidified samples since acid treatment has a significant effect on $\delta^{15}N$ values (Pinnegar and Polunin 1999). All samples were oven-dried at 60° C for 12 h. For $\delta^{13}C$ and $\delta^{15}N$ analyses, a 100 ± 10 μg portion of each ground sample was placed in a foil capsule in a numbered cell in a 96-cell plastic well-plate.

Stable isotope assays. Samples analyzed for $\delta^{13}C$ and $\delta^{15}N$ (N = 1,265) at the University of California-Davis Stable Isotope Facility represented seven potential carbon sources (N = 104 samples) and 107 consumer taxa (N = 1,161 samples) [ESM: Table 1]. The $\delta^{13}C$ reference standard was Pee Dee Belemnite (mean value: -23.83 ± 0.07 ‰, N = 262). The standard for $\delta^{15}N$ was atmospheric nitrogen (mean: 1.33 ± 0.11 ‰, N = 262). Sulfur isotope ($\delta^{34}S$) assays were accomplished at the USGS Menlo Park, California, Stable Isotope and Tritium Laboratory using Canyon Diablo meteoric triolite as the reference standard (analytical precision to < 0.2 ‰).

Trophic guilds. Seven potential carbon source guilds included: near-surface POM (> 125 μm, < 335 μm), near-surface holoplankton (> 335 μm), near-bottom POM, near-bottom holoplankton, bottom sediment, and two pelagic macroalgae (*Sargassum* spp. and *C. liniformis*). Deef-reef consumers were classified into 11 empirically-defined guilds [ESM: Table 1] based on food habits (Weaver et al. 2002): *Sargassum* associates (SA), epipelagic microvores (EMI), epipelagic intermediate macrovores (EIM), benthic particulate feeders (BPF), benthic miniparticulate feeders (BMF), benthic planktivores (BPV), benthic deposit feeders (BDF), benthic microvores (BMI), benthic omnivores (BOM), benthic intermediate macrovores (BIM), and benthic macrovores (BMV).

Two-way ANOVA was used to test the hypothesis that four POM and holoplankton carbon sources did not differ from one another by size fraction or surface versus bottom. One-way ANOVA was performed to test significant $\delta^{13}C$ differences between consumer guilds. If necessary to satisfy ANOVA assumptions of normality and homogeneity of variances, data were log transformed. If ANOVA results indicated significant treatment effects, a Tukey's multiple comparison tested for differences among individual treatments (Sokal and Rolfe 1995). The criterion of statistical difference for all tests was $p < 0.05$. Trophic spectra (Monteiro et al. 1981) were plotted for selected entities (if N ≥ 20) as $\delta^{13}C$ frequency histograms.

Results

Among 114 producers and consumers (N = 1,265 specimens) analyzed for $\delta^{13}C$ and $\delta^{15}N$, sample size of N ≥ 20 was achieved for 21 entities. Only four sediment samples had sufficient mass of organic matter for $\delta^{13}C$ analysis. Ten tissue samples of the benthic planktivorous fish, *Pronotogrammus martinicensis* were analyzed, returning a mean $\delta^{34}S$ value of 19.93 ± 0.55.

Two-way ANOVA contrasts of data for organic carbon source guilds revealed no statistical differences in mean $\delta^{13}C$ between four plankton guilds (near-surface versus near-bottom, and POM versus net holoplankton). Therefore, all four plankton guilds were combined into one overall 'holoplankton' group representing the primary carbon source available to OCS consumers in this study. A t-test revealed no significant difference ($p < 0.05$) in mean $\delta^{13}C$ between the combined holoplankton group and bottom sediment. However, bottom sediment was not grouped with holoplankton as a common carbon source, since it remains unknown if sedimentary carbon becomes resuspensed and available to consumers at depth on the OCS. No significant difference was found between $\delta^{13}C$ means for *Sargassum* spp. and *C. liniformis*, which were accordingly grouped into a single macroalgae producer guild. However, there was a significant difference ($p < 0.001$) in $\delta^{13}C$ data between holoplankton (mean $\delta^{13}C$ = -19.35 ± 1.16‰) and macroalgae (mean $\delta^{13}C$ = -16.41 ± 2.13‰), readily

distinguishing these two different OCS carbon sources (Fig. 1).

Across all consumer samples, $\delta^{13}C$ values ranged from -9.29 to -22.00 ‰, corresponding with the range determined for holoplankton and macroalgae combined (-12.62 to -24.18 ‰), allowing for trophic enrichment. No ^{13}C consumer or sediment signatures indicative of terrestrially-derived POM ($\delta^{13}C \leq$ -26 ‰) (Shultz and Calder 1976) were obtained.

One-way ANOVA contrast in $\delta^{13}C$ data for six benthic invertebrate consumers revealed that the particulate-feeding gorgonian *Nicella* sp. was statistically distinct (mean $\delta^{13}C$ = -15.01 ± 2.17 ‰) from the other five invertebrate consumers analyzed (overall mean $\delta^{13}C$ = -17.29 ± 0.90 ‰) (Table 1). One-way ANOVA contrast of the 11 empirically-defined consumer guilds resolved them into three statistically distinct ($p < 0.05$) consumer groups via $\delta^{13}C$ data, with the BMV and BMF guilds forming two single-guild groups, distinct from a large group comprised of nine guilds of intermediate predators-

Benthic invertebrate consumer	**N**	**$\delta^{13}C$±S.D. (‰)**	**$\delta^{15}N$±S.D. (‰)**
Nicella sp.	30	-15.01±2.17*	9.45±0.55
Argopecten sp.	30	-17.59±0.51	7.17±0.32
Cidaris rugosa	8	-18.93±0.83	8.93±0.53
Plesionika longicauda	17	-16.79±0.48	9.40±1.06
Portunid crab	30	-17.02±0.54	11.10±0.46
Crangon sp.	10	-16.75±1.41	10.22±0.87

Table 1. Comparative $\delta^{13}C$ signatures for six benthic invertebrate consumer entities. * Statistically distinct, $p < .05$.

omnivores (hereafter termed the IPO group) (Fig. 1). Within the IPO group, two guilds, the SA guild and the BPV guild, formed statistically distinct subgroups.

Two of the three major consumer groups form a 1-2-step carbon chain based on holoplankton (Fig. 1). This chain includes all consumer guilds except for the distinctive BMF guild/group, which appears linked to a distinct macroalgal carbon source via a 1-step trophic chain along a parallel $\delta^{13}C$:$\delta^{15}N$ trophic enrichment regression line (Fig. 1).

Statistical contrasts revealed a very small (0.4 ‰), but significant difference between $\delta^{13}C$ means for all pelagic consumers versus all benthic consumers, and between means (Δ = 0.2 ‰) for all epipelagic microvores (EMI) versus all benthic microvores (BMI). However, these differences are negligible compared to the >3.0 ‰ difference in $\delta^{13}C$ up the $\delta^{15}N$ progression, between the holoplankton and

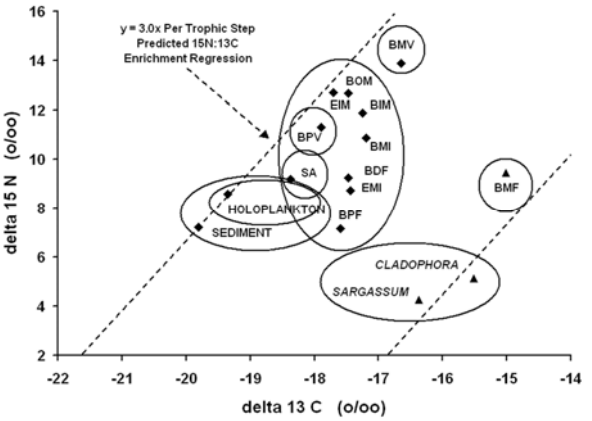

Figure 1: Producer and consumer groups and subgroups (ellipses) defined by mean $\delta^{13}C$ signatures. Abbreviations identify empirically-defined consumer guilds (points are mean $\delta^{13}C$ signatures per guild).

macroalgal trophic chains (Fig. 1). Thus, benthic versus pelagic consumer ^{13}C enrichment (McConnaughey and McRoy 1979; Fry and Sherr 1984; France 1995; Jennings et al. 1997; Pinnegar and Polunin 2000; Hobson et al. 2002) alone cannot account for the enriched $\delta^{13}C$ signatures of *Nicella* or individuals in other benthic taxa (Table 2).

Trophic spectra for most NEGOM deep-reef consumers displayed a sharp unimodal $\delta^{13}C$ peak corresponding with phytoplankton-derived carbon, as in the planktivorous fish *P. martinicensis* (-17.71 ± 0.37 ‰) (Fig. 2). A few consumers such as the planktivorous fish, *Hemanthias vivanus*, displayed a broad $\delta^{13}C$ spectrum (-16 to -22 ‰), but a similar mean (-18.25 ± 1.15 ‰) (Fig. 2). The miniparticulate feeding gorgonian, *Nicella*, displayed a distinctive strongly bimodal $\delta^{13}C$ spectrum (Fig. 2), one mode centered at -16 ‰ (phytoplankton carbon), another at -13 ‰ (macroalgal carbon). Trophic selectivity is

Trophic guild	**N**	**Mean $\delta^{13}C$ range**
Miniparticulate feeders (*Nicella* sp.)	17	-9.19 to 15.87
Sargassum associates	1	-15.92
Epipelagic intermediate macrovores	4	-14.43 to -15.69
Benthic microvores	5	-12.90 to -15.00
Benthic deposit feeders	1	-15.68
Benthic intermediate macrovores	17	-14.12 to -15.95
Benthic macrovores	11	-15.00 to -15.97

Table 2. Consumer guilds containing taxa with individuals (n) yielding notably enriched (≥ -16 ‰) $\delta^{13}C$ signatures.

evident in the comparative spectra of these three syntopic reef-crest particulate feeders, which all feed within a meter of the reef surface. The broad $\delta^{13}C$ spectrum of *H. vivanus* indicates consumption of both POM and larger holoplankton. In contrast, *P. martinicensis* appears to be a zooplankton specialist, while *Nicella* appears to shift between holoplankton versus macroalgae-derived particles, utilizing a carbon source not selected by its competitors.

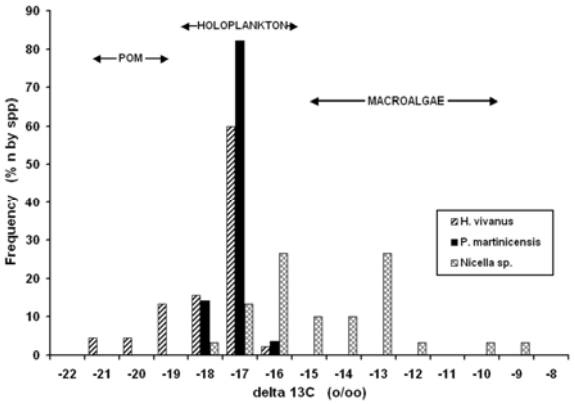

Figure 2. Comparative ^{13}C isotopic spectra (versus probable carbon sources) for three deep-reef particulate feeders: *Hemanthias vivans* (hatched bars) and *Pronotogrammus martinicensis* (black bars), and the gorgonian *Nicella* sp. (cross-hatched bars).

NEGOM OCS carbon sources. No highly depleted $\delta^{13}C$ signatures typical of terrestrial plant carbon (e.g., $\delta^{13}C \leq$ -26 ‰) were observed among the analyzed consumer entities. Additionally, $\delta^{34}S$ values determined for 10 specimens of the benthic planktivorous fish, *P. martinicensis* ranged from +19.20 to +20.89 ‰, (mean +19.93 ‰). These values lie within the range of $\delta^{34}S$ values (+16.6 to +20.3 ‰) previously reported for seawater sulfate, marine plankton, oceanic fish, and offshore consumers (Rees et al. 1978; Peterson et al. 1985, 1986; Fry 1988; Hesslein et al. 1993), providing no evidence of terrestrial sulfur from either C3 plant detritus ($\delta^{34}S$ +4.7 ‰, Peterson and Howarth 1987) or deposited freshwater sediment ($\delta^{34}S$ -14 ‰, France 1995). These ^{13}C and ^{34}S results confirm earlier findings from larval fishes (Thayer et al. 1983), bottom sediments (Shultz and Calder 1976; Eadie et al. 1978), indicating that terrestrial organic input is limited to NEGOM estuarine and nearshore waters (Gearing et al. 1977; Fry and Sherr 1989).

However, findings herein challenge the fundamental assumption that phytoplankton is the sole source of fixed carbon for offshore food webs (Fry and Sherr 1984). Enriched $\delta^{13}C$ signatures ($\geq$ -16 ‰) for individuals in 23 consumer taxa in six guilds (Table 2) provide evidence of a second OCS carbon source. Given a ^{13}C turnover time in fish tissue of months to years (Hesslein et al. 1993), sustained selective feeding on an enriched food source would be required to be reflected in signatures from tissues analyzed (Post 2002). The bimodal spectrum of *Nicella* (Fig. 2) is particularly instructive. The ^{13}C depleted mode at -16 ‰ corresponds with intake of phytoplankton-chain derived particles (holoplankton mean $\delta^{13}C$ = -19.35 ‰), allowing for 1-step trophic enrichment. The enriched mode centered upon –13 ‰ (Fig. 2) suggests intake of particles from a macroalgal source, e.g, *Sargassum* (mean $\delta^{13}C$ = -16.37 ‰) and *C. liniformis*. (mean $\delta^{13}C$ = -15.51 ‰), after one step enrichment.

While oceanic phytoplankton is ubiquitous in time and space, *Sargassum* is discontinuously distributed in both regards. Thus, carbon contributed to tropical and temperate oceanic ecosystems by *Sargassum* would typically be swamped (Parr 1939) in the long-term integrated ^{13}C data presented by most consumer species. Only individuals which have fed on items carrying *Sargassum* carbon for an extended period would display correspondingly enriched $\delta^{13}C$ signatures. A specialist using a trophic switching strategy would display two dominant modes (Monteiro et al. 1981), as seen in *Nicella*. This suspensivore, which inhabits the current-ward crests of deep reefs, appears to selectively switch between particulates derived from macroalgae, or alternatively from plankton-based particulates. Approximately 45 % of the *Nicella* $\delta^{13}C$ frequency distribution (signatures from -9 to –15 ‰) (Fig. 2) appears attributable to macroalgal carbon. A related Mediterranean gorgonian, *Eunicella stricta,* is a specialist deriving most of its carbon from algae and the seagrass *Posidonia*, a refractory carbon source not otherwise utilized by many consumers (Dauby 1989). The $\delta^{13}C$ value reported for *E. stricta* (-14.2 ‰) s close to the mean herein for *Nicella* sp. (-15.01 ± 2.17). Ribes et al. (2003) reported that the gorgonian *Leptogorgia sarmentosa* feeds on a broad range of suspended particles, from nanoeukaryotes < 3.6 µm to zooplankton >100 µm. This species specializes seasonally, utilizing food sources as a function of availability, with a summer minimum in detrital POM use. However, POM accounted for 30.4% of total assimilated carbon. Another gorgonian, *Paramuricea clavata*, derived 48% of its food spectrum from detrital POM (Ribes et al. 1999). A pattern of seasonal selectivity or switching could result in the bimodal $\delta^{13}C$ trophic spectrum of *Nicella* sp.

Parr (1939) estimated the biomass of *Sargassum* in the Sargasso Sea as 3.6-10.0 x 10^9 kg, three orders of magnitude less than estimated phytoplankton biomass for that region. Despite this fact, and the refractory nature of brown algae (Montgomery and Gerking

1980), it seems unlikely that such a biomass of carbon would go unutilized in the world ocean. Present findings suggest it does not. Evidence of selective use of pelagic macroalgal carbon by one deep-reef gorgonian suggests that other OCS reef consumers may depend in part upon this carbon source. Accordingly, their spatial occurrence and abundance may be tied to the predictable concentration of macroalgae along major ocean surface features.

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Bacterial degradability of dissolved organic carbon in coral mucus

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Abstract
Bacterial decomposition of dissolved organic carbon (DOC) in coral mucus was investigated under dark conditions for 7 months to reveal what percentage of the coral-derived DOC is rapidly utilized by bacteria, and conversely, what percentage remained for a long term as refractory organic matter. During the first week, 38%-47% of DOC released from the hermatypic corals *Acropora pulchra* and *Porites cylindrica* were mineralized by bacteria collected from natural reef water. The bacterial abundance dramatically increased from the order of 10^3 to 10^6 cells ml^{-1} during the 1 week. Some part of the remaining organic C at 1 week was slowly decomposed over 3 months, but further degradation was not observed thereafter. Finally, 32%-44% of the initial DOC was not mineralized over 7 months. These results suggest that, under dark conditions, DOC in coral mucus is not completely degraded by free-living bacteria and contributes to long-term C fixation as refractory organic matter.

Keywords: coral, mucus, bacteria, mineralization, refractory organic matter

Introduction

It is well known that coral colonies release organic matter to the ambient seawater as dissolved and particulate organic matter (DOM and POM, respectively) (Tanaka et al. 2008). The coral-derived organic matter is often collectively referred to as mucus. The coral mucus has been regarded as ecologically important: bacterial aggregation was found in coral mucus (Ducklow and Mitchell 1979) and coral exudates actually enhanced the growth of pico- and nanoplankton (Ferrier-Pagès et al. 2000). Wild et al. (2004a, b) showed that a part of coral mucus was rapidly mineralized into CO_2 by bacteria in the reef sediment. The organic matter released from corals has been considered to be a good source of energy for microorganisms and to be completely and rapidly mineralized into CO_2 in the coral reef ecosystem.

However, some studies proposed negative ideas on the bacterial decomposition of coral mucus. Vacelet and Thomassin (1991) observed that coral mucus of high molecular weight (more than 6000−8000) was not completely degraded by bacteria and eukaryotes even after 21 d. Krupp (1984) and Coffroth (1990) concluded that coral mucus itself is a material of low nutritional value for reef organisms and that the property to collect organic detritus and to serve as sites of aggregation for microorganisms is a more important trophic role of coral mucus. More recently, Ritchie (2006) and Chen et al. (2007) found antibacterial substance from coral mucus. These studies suggest that some part of the organic matter released from corals is not rapidly mineralized by bacteria but remain as relatively refractory organic matter.

Therefore, no previous studies have revealed what % of the coral-devied organic matter is rapidly decomposed by bacteria and what % is not. The purpose of this study is to quantitatively investigate bacterial degradability of coral mucus for a long term. Dissolved organic matter in pure coral mucus was put under dark and the change in organic C concentration due to bacterial mineralization was observed over 7 months.

Material and methods

The experiment was performed using two hermatypic corals *Acropora pulchra* and *Porites cylindrica*, which were collected on the reef crest of Shiraho reef in Ishigaki Island (24° 21′-31′ N, 124° 4′-16′ E), Japan in Jul 2007. In Shiraho reef, the reef crest is developed roughly parallel to the shoreline at a distance of ca. 850 m. The corals were collected ca. 700 m offshore, where the depth was ca. 0.5 m at low tide. Six coral branches were cut from several colonies, and put in two glass bottles (i.e., three branches per bottle) for each coral species (total four bottles). The glass bottles (900 ml) contained 300 ml of artificial seawater (ASW). Surface area of each coral branch was 24-53 cm^2 (average 41 cm^2). The water volume was enough to cover the corals. To minimize organic matter contamination in the prepared ASW,

sodium chloride (NaCl), magnesium sulfate ($MgSO_4$), potassium chloride (KCl), and calcium chloride ($CaCl_2$) were combusted at 450˚C for 3 h in advance.

After the collection of the coral branches, the glass bottles were immediately carried to the laboratory. During the transportation, the corals produced much mucus due to the stress of gentle water turbulence in the bottles. Temperature of the seawater in the bottles was same as that of in situ reef water (29-30˚C). In the laboratory, the coral branches were taken out of the bottles. The duration of mucus production was 1 h. 400 ml of ASW was added to each bottle to dilute the produced mucus, and 5 ml of in situ reef seawater was also added to inoculate bacteria in the water column. The sample seawater (total 705 ml for each bottle) was then filtered with pre-combusted Whatman GF/F filters (pore size: 0.7 μm) to remove particulate organic matter (POM) including flocculated coral mucus and expelled zooxanthellae. The filtrate was put in pre-combusted glass bottles (1 L), and the bottles were placed under dark to observe mineralization of DOM in the filtrate. To make same temperature condition, the dark room was kept at 20˚C.

Sampling of the filtrate was conducted at 0, 2, 7, 30, 90, and 220 days of the dark incubation. 15 ml of the seawater was taken into pre-combusted glass ampoules to measure the concentrations of total organic C (TOC) and inorganic nutrients. The ampoules were immediately sealed and stored at -20˚C until analysis. 10 ml of the seawater was taken into acrylic tubes and fixed with pre-filtered formalin (final concentration 2%, v/v) to measure bacterial abundance.

The concentration of TOC was measured by the high temperature catalytic oxidation method (HTCO) using TOC-5000 (Shimadzu). Milli-Q water was used for blank and coefficients of variance for the analysis were <5%. Dissolved inorganic N (DIN: NO_3^-, NO_2^-, NH_4^+) and PO_4^{3-} concentrations were quantified with a nutrient analyzer AACS-III (BRAN+LUEBBE). Analytical error of the nutrient measurement was <0.02 μmol l^{-1}. Duplicate samples from each bottle were analyzed for TOC and inorganic nutrients.

The abundance of bacteria fixed by formalin was counted with an epifluorescence microscopy after staining with SYBR Gold. Bacterial aggregates were disintegrated by weak ultrasonic treatment for 20-30 s. At least 400 bacterial cells were counted from 20-25 fields, except for the samples at t = 0 d. For the samples at t = 0 d, 80-170 cells were counted from >40 fields, due to the small bacterial abundance.

The production rate of DOM (R: μmol cm^{-2} h^{-1}) by the corals was calculated from the organic matter (C: μmol l^{-1}) accumulated in seawater.

$$R = C\,V\,S^{-1}\,T^{-1} \quad (1)$$

where V, S and T are seawater volume (l), coral surface area (cm^2), and the duration of mucus production (h), respectively. Coral surface area was determined by aluminum-foil method (Marsh 1970). ASW used for the experiment initially had minor contamination of organic C (4.8 μmol l^{-1}), compared to the DOC produced by the corals (see below), thus, the C amount was subtracted from all the measured concentrations of TOC at each sampling time, supposing that the initially-contained organic C did not change during the experiment.

Bacterial growth efficiency (E) during the dark incubation was calculated as follows.

$$E = \Delta BC\,(\Delta TOC)^{-1} \quad (2)$$

ΔBC and ΔTOC are an increased bacterial C (μmol l^{-1}) and a decreased TOC (μmol l^{-1}) during a period, respectively. Bacterial C was estimated from determined bacterial number using the conversion factor of 15-30 fg C per bacterial cell (Fukuda et al. 1998).

Results and discussion

DOC accumulated in ASW by the range of 80-97 μmol l^{-1} (Table 1). The release rates normalized to the coral surface area were 400-440 nmol cm^{-2} h^{-1} for *A. pulchra* and 510-650 nmol cm^{-2} h^{-1} for *P. cylindrica* (range of duplicate bottle incubations) (Table 1). The release rates were comparable to those of *Acropora millepora* under the stress of air

Table 1 Initial conditions of the decomposition experiment. 'Conc.' means DOC concentration accumulated in the seawater. Release rates are normalized to the coral surface area. DIN includes nitrate, nitrite, and ammonium. Error bars indicate standard error of analysis

	DOC		DIN	PO_4^{3-}	Bacteria
	Conc. (μmol L^{-1})	Release rates (nmol cm^{-2} h^{-1})	(μmol L^{-1})	(μmol L^{-1})	(10^3 cells ml^{-1})
Acropora 1	80	444	0.86	0.24	4.4±3.0
Acropora 2	84	399	0.92	0.23	2.9±1.5
Porites 1	85	514	1.0	0.19	3.9±3.0
Porites 2	97	650	1.2	0.19	7.8±6.3

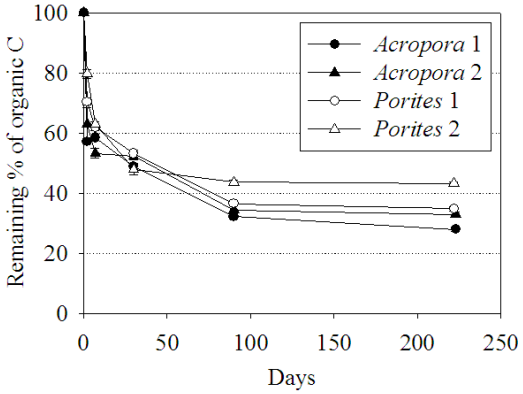

Fig. 1 Bacterial mineralization of dissolved organic carbon released from the four corals. Data are shown as remaining % of the initial concentrations, which ranged from 80-97 μmol l^{-1}. Error bars indicate standard deviation of analysis.

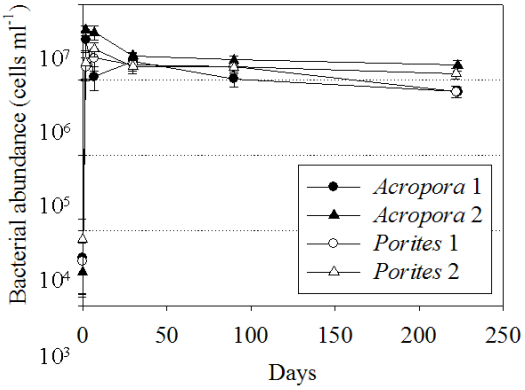

Fig. 2 Bacterial abundance during the organic matter decomposition. Data are indicated as mean ± standard error of analysis.

exposure (980±660 nmol cm^{-2} h^{-1}; Wild et al. 2005). Using the same coral species as the present study, Tanaka et al. (2008) measured DOM and POM release rates from the submerged corals as naturally as possible. The observed DOC release rates were 380 and 340 nmol cm^{-2} d^{-1} for *A. pulchra* and *P. cylindrica*, respectively (Tanaka et al. 2008). Assuming that DOC was continuously released in natural condition at daytime, the release rates of 380 and 340 nmol cm^{-2} d^{-1} would be one order of magnitude lower than those observed in the present study (400-650 nmol cm^{-2} h^{-1}). Wild et al. (2005) also compared the release rates of particulate organic C from *A. millepora* between the corals in air exposure and in a submerged condition, and showed that the release rates under the stress (980±660 nmol cm^{-2} h^{-1}) were about 10 times higher than those in the submerged.

This is the first study which observed the mineralization of DOC released from corals in the time scale of several months. The concentration of TOC dramatically decreased by the range of 32-39 μmol l^{-1} during the first 1 week (Fig. 1). Decreased % was very similar among the four incubations (37%-41%; Table 2). As the organic C was decomposed, NH_4^+ concentration increased from 0.8-0.9 μmol l^{-1} to 5.1-6.1 μmol l^{-1} during the 1 week, indicating that DON released from the corals was simultaneously mineralized. Bacterial abundance increased within the first 2 d by a factor of 1000, i.e. from 10^3 to 10^6 cells ml^{-1} (Fig. 2). Bacterial growth efficiency during the 2 d was calculated to be 12%-37% for *A. pulchra* and 7%-21% for *P. cylindrica*. These are usual values for the growth efficiency of marine bacteria in log phase (4%-30%; Carlson and Ducklow 1996), suggesting that labile organic matter in the coral mucus had similar degradability to that produced in other marine ecosystems, mainly by phytoplankton.

After 1 week, the concentration of TOC slowly decreased until Day 90 (Fig. 1). Average remaining % after 90 d was 37% (Table 2). First order decay constants (*k*) during 7-90 d were 0.004-0.007 d^{-1}, which were one order of magnitude lower than those of the first 1 week (0.066-0.077 d^{-1}; Table 2). After 90 d, TOC concentration decreased only by 1%-4% during the next 130 d (Fig. 1). Average remaining % of organic C after 220 d was 35% (Table 2). *k* values during 90-220 d were 0.0001-0.001 d^{-1} (Table 2). Considering that the decay constants were distinctly different among the three phases (i.e., 0-7 d, 7-90 d, and 90-220 d), the change in TOC concentration in Fig. 1 was not dependent on the concentration but on the different degradability of the organic matter during each phase. It can be defined that the organic matter decomposed during 0-7 d, 7-90 d, and 90-220 d is relatively labile fraction (F_L), semi-labile fraction (F_S), and refractory fraction (F_R), respectively. Supposing that the three defined fractions (F_L, F_S, and F_R) were only mineralized during each period (i.e., 0-7

Table 2 Remaining % of organic C at each sampling time. *k* values are first order decay constants during each decomposition phase.

	Period (d)	Remaining %	k (d^{-1})
Acropora 1	0-7	100 → 59	0.077
	7-90	59 → 32	0.007
	90-223	32 → 28	0.001
Acropora 2	0-7	100 → 53	0.090
	7-90	53 → 35	0.005
	90-223	35 → 33	0.0004
Porites 1	0-7	100 → 62	0.069
	7-90	62 → 37	0.006
	90-222	37 → 35	0.0003
Porites 2	0-7	100 → 63	0.066
	7-90	63 → 44	0.004
	90-222	44 → 43	0.0001

d, 7-90 d, 90-220 d), the percentage of each fraction can be calculated from the decreased % in Table 2: F_L, F_S, and F_R accounted for 37%-47%, 19%-26%, and 32%-44%, respectively (on average, F_L: 41%, F_S: 22%, F_R: 37%). Adding up F_L and F_S, about 60% could be mineralized by the bacteria within 3 months.

The present study has quantitatively shown that a part of the organic matter released from the corals is not mineralized into CO_2 by free-living bacteria in the reef water. There existed some inorganic nutrients in the incubated seawater at the end of the experiment (t = 220 d): 7.8-10.2 μmol N l^{-1} and 0.38-0.55 μmol P l^{-1}. Thus, bacterial incorporation of the remaining organic C could not be limited by the availability of N and P. Some possibilities are suggested for the remaining organic C. First, the organic matter released was originally recalcitrant to bacterial decomposition. Using coral mucus with molecular weight larger than 6000−8000, it was observed that the mucus web was not completely degraded by bacteria and eukaryotes even after 21 d (Vacelet and Thomassin 1991). They explained that mucus excretion from coral is a defensive reaction against physical and chemical stresses, thus mucus is a poor, or even inhibiting medium for the bacterial degraders isolated from the mucus itself. Similar results were also reported by Pascal and Vacelet (1981). Krupp (1984) and Coffroth (1990) also suggested that coral mucus is a material of low nutritional value for reef organisms. More recently, Ritchie (2006) observed that mucus from *Acropora palmata* had antibiotic properties that were likely to play a role in ordering only beneficial microbial communities on the coral surface. Antibacterial substance was also found from coral mucus of *Symphyllia gigantea* (Chen et al. 2007) and from tissue extraction of some soft corals (Kelman et al. 2006). Coral colonies might routinely produce organic matter resistant to the degradation by bacterial communities in the water column.

The second possibility explaining the remaining organic matter is that coral-derived organic matter was once utilized by bacteria and converted into different organic forms, which were recalcitrant to further bacterial decomposition. Ogawa et al. (2001) reported that when marine bacteria utilized labile compounds (glucose, glutamate), they produced refractory DOM (R-DOM) that persisted for more than a year. Their result suggested that microbial processes alter the molecular structure of DOM, making it resistant to further degradation. Considering that 5%−7% of original substrate C (glucose, glutamate) were altered to R-DOM in Ogawa et al. (2001), R-DOM produced by bacteria in the present study could account for only a minor part of the remaining organic matter (Fig. 1 & Table 2), supposing that the bacteria produced R-DOM with the similar ratio. Therefore, the preservation of the organic matter in the present experiment (ca. 35%) could not be explained only by bacterial activity to produce R-DOM, but some parts of the remaining organic matter could originally have resistance against bacterial decomposition.

Wild et al. (2004a, b) pointed out the important role of bacteria in reef sediment to decompose coral mucus. They considered that a highly diverse and dense sedimentary bacterial population, adapted to the decomposition of more refractory material, can decompose coral mucus more effectively than the bacterial community in water column. Because only the bacteria in water column were inoculated in the present study, the possibility that sedimentary bacteria decompose the remaining organic matter needs to be investigated in the future. However, a part of coral-derived organic matter could diffuse and be exported to the outer ocean without settling down to the sediment, especially in the reef where water residence time is short. For example, in the study site of the present study (Shiraho reef), average residence time of the lagoon water was estimated to be 4-8 h (H. Yamano unpubl. data). Hata et al. (2002) concluded that 6%-7% of gross primary production in Shiraho reef was exported to the outer ocean as POC. Their study also suggested the export of DOC from the lagoon. The present paper showed that considerable parts of the exported DOC might remain as refractory organic matter over several months.

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NOAA Coral Reef Watch Coral Bleaching Outlook System

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Abstract. A new NOAA tropical coral bleaching outlook system has just been developed. The system includes a sea surface temperature (SST) prediction model based on NOAA's Linear Inverse Model (LIM) and a bleaching thermal stress model based on the coral bleaching HotSpot and Degree Heating Week (DHW) algorithms used in NOAA Coral Reef Watch (CRW) operational near-real-time satellite bleaching monitoring. The system forecasts regions of potential thermal stress conducive to coral bleaching ranging from one week to 18 weeks in advance.

Key words: Coral bleaching, Thermal stress, Prediction, Forecast, Outlook.

Introduction

Mass bleaching of coral reefs has occurred with increasing frequency in recent decades. The US National Oceanic and Atmospheric Administration's (NOAA) Coral Reef Watch (CRW) provides critical information to reef managers and scientists based on near-real-time satellite monitoring of thermal stress conducive to coral bleaching (Liu et al. 2006). However, many managers and scientists have requested information on the likelihood of coral bleaching months in the future.

NOAA CRW, in collaboration with the Physical Sciences Division of the NOAA Earth System Research Laboratory (ESRL), has just developed a bleaching prediction tool capable of forecasting weeks to months in the future. Using global sea surface temperature (SST) forecast models, NOAA CRW now provides outlooks of potential bleaching thermal stress, to significantly improve strategic planning and management of coral reef ecosystems. Here we introduce this bleaching outlook system and evaluate its performance by comparison with CRW operational near-real-time observations.

Bleaching Outlook System

The NOAA bleaching outlook system covers the tropical latitudes between 30ºS and 30ºN. The outlook comprises three integrated parts: sea surface temperature (SST) prediction model, coral bleaching thermal stress prediction, and coral bleaching outlook. The SST model results are currently available at 2º×2º ×weekly resolution and for lead times of 1 to 24 weeks. The seasonal bleaching outlook is created from the maximum thermal stress forecast up to 18 weeks into the future.

Sea Surface Temperature Prediction Model

The system uses the SSTs predicted by the NOAA Linear Inverse Modeling (LIM) developed by NOAA/ESRL Physical Sciences Division (Penland and Matrosova 1998; Penland and Magorian 1993; Penland 1989). LIM is a statistical derivation of the best dynamical description from the observations of a linear system and prediction are made from the derived statistical model. Penland and Sardeshmukh (1995a) and Penland and Matrosova (1998) found that the evolution of tropical SST anomalies (SSTAs) can in large part be represented as a stable, multivariate linear dynamical system maintained by stochastic forcing. From this, NOAA developed the NOAA LIM prediction model for forecasting tropical SSTAs.

Weekly 1º×1º Reynolds and Smith Optimum Interpolation SST (OISST) data (Reynolds et al. 2002 and 1994) between 30°N and 30°S during the years 1982-2006 were consolidated onto 2º×2º grids to train our model. This minor reduction in spatial resolution reduces the number of degrees of freedom one must diagnose from a fairly short data set. Then, weekly climatologies were derived and weekly SSTAs were calculated. The resulting anomalies were cast in terms of Empirical Orthogonal Functions (EOFs). Most of the variability and almost all of the predictable variability were compressed into a basis having a much smaller dimensionality than the original data. The leading 30 EOFs that contain 75% of the data are retained in the model. Using this compressed

description of the data, LIM was applied to estimate the linear operators giving the best forecast of SSTA.

Prediction of global tropical SSTAs is made by applying the statistical model described above to the observed initial SSTAs as represented by EOFs from weekly OISST. The prediction in the EOF domain is then cast back to SSTA in the geospatial domain. Both initial conditions and forecasts have smaller amplitude than the original data because, on average, only 75% of the variance was retained. To account for this reduction, predicted anomaly at a location is inflated by a factor reflecting the amount of variance retained at that location. For example, SSTAs in a location where only half the variance is retained are inflated by the square root of 2, while very little is inflated in a region that retained nearly 100% of the variance. The climatology is added to the adjusted prediction of the SST anomaly to give the SST forecast.

Bleaching Thermal Stress Prediction Model

The stress forecast methodology is similar to the CRW operational near-real-time satellite coral bleaching monitoring. In the near-real-time monitoring, the HotSpot measures the intensity of bleaching thermal stress as the difference between the observed SST at a grid point and the climatologically averaged temperature for the warmest month. Since both intensity and duration of thermal stress are important in causing bleaching, the Degree Heating Week (DHW) accumulates HotSpot values ≥1.0 ºC during the prior 12 weeks (Liu et al. 2006). DHWs of 4 ºC-weeks is likely to cause significant coral bleaching, and 8 ºC-weeks or above is likely to cause widespread severe bleaching and significant mortality (Liu et al. 2006).

Similarly, in the stress forecast, the HotSpot prediction is calculated as the difference between the model-predicted SST and the climatologically averaged OISST temperature for the warmest month. In deriving DHW prediction, we found that a threshold of 0.5 ºC of predicted HotSpot for accumulating DHW prediction yielded the best agreement between the predicted DHW and CRW's near-real-time DHW observation. This lower threshold is a consequence of reduced amplitude of SST variation intrinsic to the LIM model and a reduced spatial and temporal resolution in the outlook relative to CRW near-real-time observations. Weekly bleaching thermal stress predictions are produced for the upcoming 18 weeks.

Coral Bleaching Outlook Model

A coral bleaching outlook algorithm was developed to derive a bleaching risk level from the predicted thermal stress. Three levels of bleaching risk are defined based on the relationship between the hindcast HotSpot and DHW for 2001-2007 and the HotSpot and DHW observations from archived 2001-2007 near-real-time satellite data. The three levels are color-coded on an outlook map (Fig. 1) and defined as:

Potential Bleaching (light-orange):
$HS_{pred} > 0$
Potential Widespread Bleaching (orange):
$HS_{pred} > 0.5$
Potential Severe Bleaching (brown):
$HS_{pred} > 0.5$ and $DHW_{pred} > 4$

where, HS_{pred} and DHW_{pred} are HotSpot and DHW predictions, respectively. A seasonal outlook of bleaching potential for the upcoming 3 to 4 months is based on the maximum composite of weekly outlooks in the predicted seasonal time period.

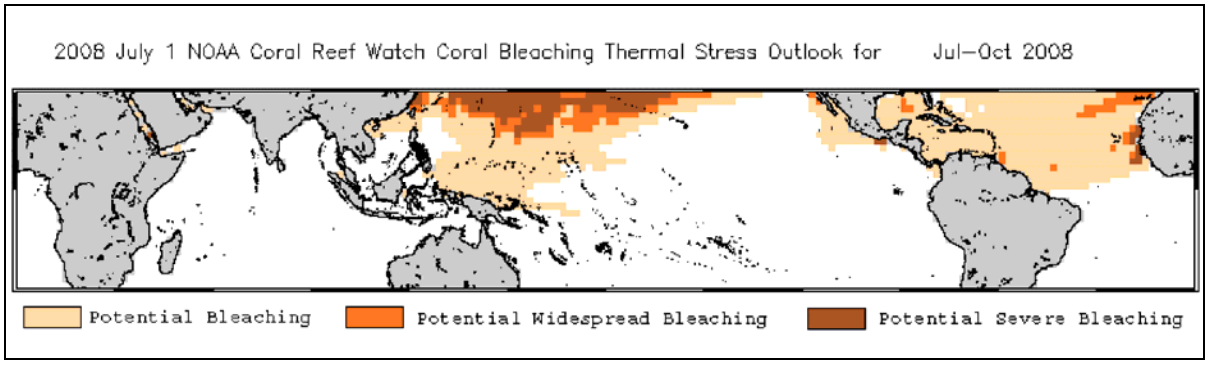

Figure 1: Seasonal bleaching outlook for July-October 2008 produced on July 1, 2008

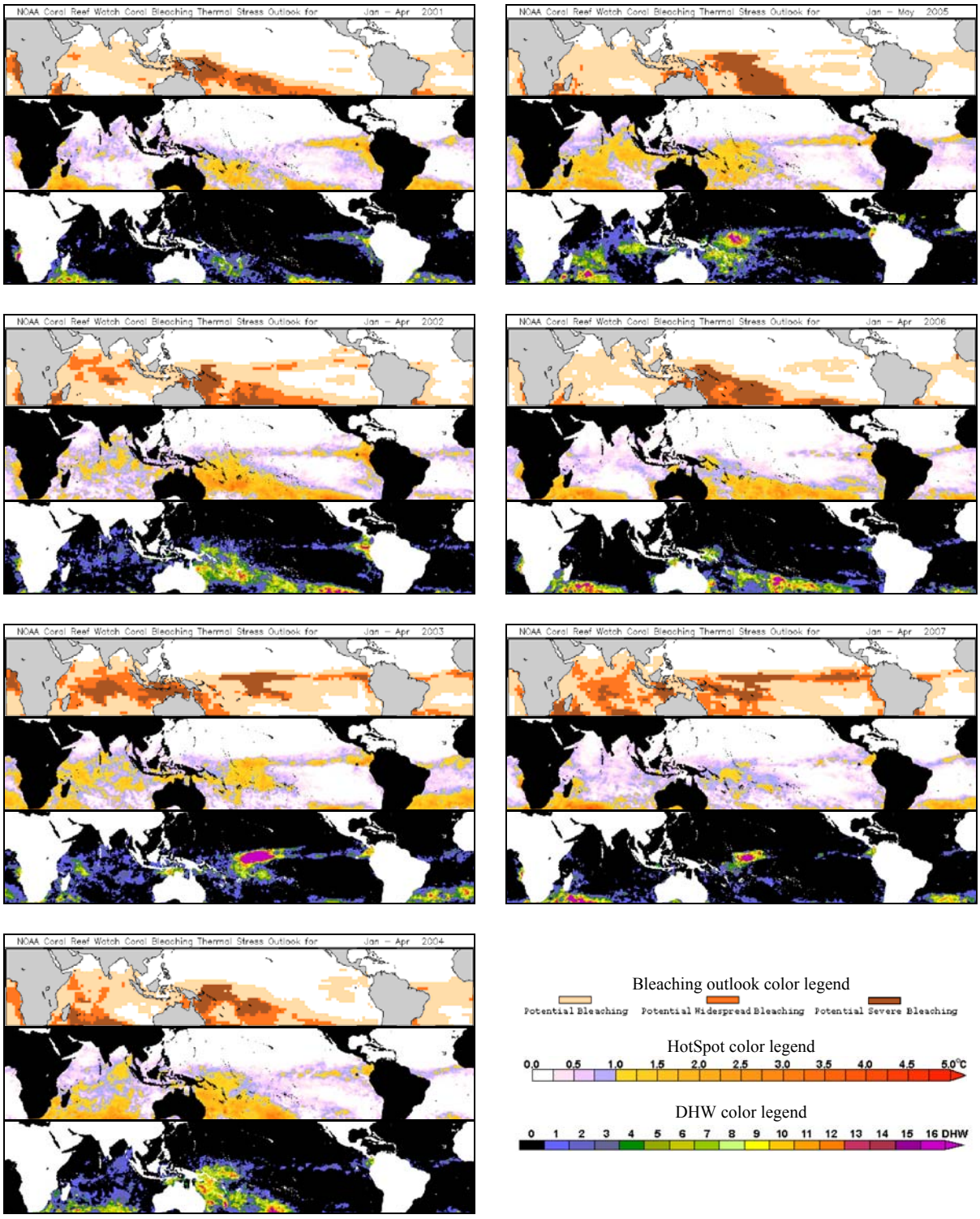

Figure 2: Hindcast austral bleaching season (January-April) seasonal bleaching outlooks for 2001-2007 (top panel of each three-panel set one for each year) and the maximum composites of the CRW near-real-time twice-weekly satellite HotSpot and DHW observations for the corresponding time periods (middle and bottom panel of each three-panel set).

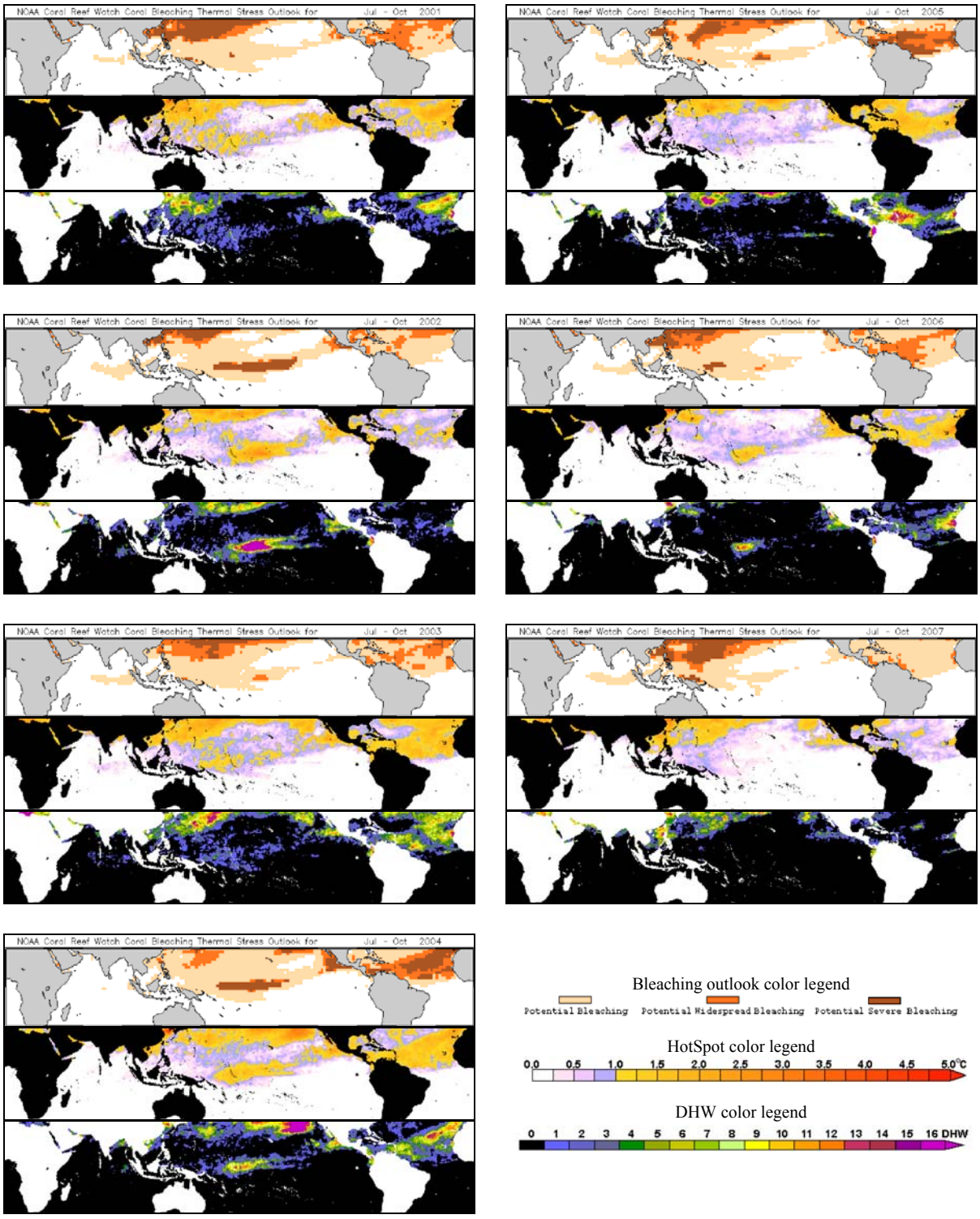

Figure 3: Hindcast boreal bleaching season (July-October) seasonal bleaching outlooks for 2001-2007 (top panel of each three-panel set one for each year) and the maximum composites of the CRW near-real-time twice-weekly satellite HotSpot and DHW observations for the corresponding time periods (middle and bottom panel of each three-panel set).

Analysis of Seasonal Bleaching Outlook Products

We have examined the accuracy of the seasonal bleaching outlook product during a 7-year set of boreal and austral bleaching season predictions. Seasonal bleaching outlook maps for January-April and July-October of 2001-2007 are shown (Fig. 2-3)

together with the maximum composites of the CRW operational near-real-time twice-weekly HotSpot and DHW observations for the corresponding time periods. This corresponds to seven full years during which has operated its Here we compare the outlook and monitoring data for the Caribbean, Great Barrier Reef (GBR), and Northwestern Hawaiian Islands (NWHI) during known large-scale bleaching events.

The general pattern and intensity of the seasonal bleaching outlooks match well with the HotSpot and DHW observations. For the GBR region (Fig. 2), the 2002 mass bleaching event (Oliver *et al.* 2008) is well captured in the seasonal outlook. The outlook correctly predicts low to mild stress years at the GBR for 2001, 2003, 2005, and 2007, but over-estimates the thermal stress in the GBR for the 2006. For the Caribbean region (Fig. 3), the outlook performs well for all seven years, including the 2005 mass coral bleaching event (Wilkinson and Souter 2008). HotSpot and DHW observations show that in all years, high thermal stress was seen in the northwestern Pacific Ocean near the NWHI (Fig. 3). Mass coral bleaching occurred in the NWHI 2002 (Aeby et al. 2003) and 2004 (Kenyon and Brainard 2006), suggesting the outlook underestimated the stress for these two events. However, the outlook tended to overestimate for other years where thermal stress was comparatively low.

Discussion

The comparison for 2001-2007 shows that the seasonal bleaching outlooks generally match the HotSpot and DHW observations. The outlook performs best in the Caribbean and GBR regions, but does not perform as well in the NWHI. This may be caused by relatively low variance explained by the leading 30 EOFs around the NWHI, but we are continuing to investigate. Also, unlike the Caribbean and GBR, masses of warm water are not constrained by land-masses and may easily miss these small islands. Examination of weekly predictions (not shown) also indicates that these are able to predict the development and dissipation of thermal stress during mass coral bleaching events, including the record-breaking 2005 bleaching in the Caribbean.

As with any forecast model, both the variance and accuracy decline with longer lead times. In the case of this outlook product, it tends to increasingly underestimate the thermal stress at longer lead times. It also seems to develop unrealistically high thermal stress at short lead times near the end of the bleaching season. These suggest that predicted HotSpot values may need to be calibrated as a function of lead time. NOAA CRW will continue to evaluate this new prediction system to improve the accuracy for coral reefs globally.

This new NOAA bleaching outlook system has been available at CRW's website, http://coralreefwatch.noaa.gov, since July 2008. It is updated weekly to provide coral reef managers and researchers critical information on large-scale bleaching thermal stress events weeks to months in advance. Similar to seasonal hurricane outlooks, the outlook product does not to provide exact predictions at any particular reef locations, but provides general patterns of potential bleaching, enabling managers and scientists to prepare in advance.

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Recovery of *Diadema antillarum* and the potential for active rebuilding measures: modelling population dynamics

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Abstract. Recent studies suggest that population recovery of the long-spined sea urchin *Diadema antillarum* could help to reverse the phase shift observed on Caribbean coral reefs by reducing macroalgal cover and promoting coral recruitment. Interest has arisen around the potential for active rebuilding measures such as hatchery release and translocations to aid and increase the slow and patchy population recovery so far witnessed since the 1983 mass mortality event. We developed a population dynamics model for *D. antillarum* to gain insight into the key processes preventing population growth and assess the effectiveness of proposed rebuilding strategies. The model accounts for compensatory density-dependence in recruitment and growth and depensatory density effects associated with fertilization success, a possible refuge function of the adult spine canopy for juveniles and 'cultivation' of preferable habitat by grazing. Results show that the population can exist at low and high-abundance equilibria associated with high and low macroalgal abundance respectively. The switch to high abundance is hampered by high juvenile mortality due to the absence of refuges. Restoration may be aided by release of large urchins and by manipulation of macroalgal cover. Experimental work is suggested to test process models and quantify functional relationships used in this exploratory analysis.

Key words: Allee effect, cultivation effect, predation refuge, habitat modification, active rebuilding

Introduction

The long-spined sea urchin *Diadema antillarum* was once abundant on coral reefs of the Caribbean region, fulfilling a role as the keystone herbivore of macroalgae (Sammarco 1982; Carpenter 1986). Its functional extinction following the mass mortality event of the early 1980s had a profound effect on coral reef community composition and is thought to have played a major role in the subsequent phase shift from states dominated by live coral to states dominated by macroalgae (Aronson and Precht 2000). Recent studies have shown that population recovery of *D. antillarum* has the potential to reverse the phase shift, reducing macroalgal cover and promoting coral recruitment (Edmunds and Carpenter 2001; Carpenter and Edmunds 2006). Widespread population recovery of *D. antillarum* may therefore be considered as a tool with which to aid coral reef restoration in the Caribbean.

While in some locations, *D. antillarum* abundance has recovered markedly (Carpenter and Edmunds 2006); in others the population has persisted at a low level of abundance (Lessios 2005; Chiappone et al 2002). The mechanisms underlying differential recovery and the potential for active rebuilding measures remain poorly understood. Hypotheses relating to the negative impact of low population density on population growth may explain the slow and patchy recovery that has been observed across the region (Lessios 1988). *D. antillarum* is a dioecious species with broadcast spawning, a long larval stage and limited adult dispersal (Lessios 1988). Despite their long spines, *D. antillarum* are susceptible to predation by around 15 fish species and multiple invertebrates with the greatest risk being to smaller size classes (Carpenter 1984; Clemente et al 2007). Direct negative effects of low population density, referred to as Allee effects may be responsible for a reduction in fertilization success, larval settlement and post-settlement survival (Bak 1985; Levitan 1991; Miller et al 2007). At low population density the chances of adult urchins spawning synchronously and at close enough proximity to allow for successful fertilization is greatly reduced (Levitan 1991). Larvae in the water column may take settlement cues from conspecifics and it is thought that the spine canopy of adult urchins may act as an important predation refuge for susceptible juveniles (Nishuzaki and Ackerman 2007; Miller et al 2007). Both larval settlement and post-settlement survival may be further reduced at low population density through an indirect effect which we will refer to as the cultivation effect.

As prolific grazers and bioeroders, *D. antillarum* are associated with habitats low in macroalgal cover and high in reef topographic complexity (Lee 2006). Evidence suggests that surfaces clear of macroalgae are preferred for larval settlement (Bak 1985) and we hypothesize that reefs that lack macroalgae and have greater topographic complexity will naturally have more reef cracks and crevices that provide effective predation refuges. Few studies have set out to test hypotheses relating to Allee and cultivation effects and none have aimed to quantify their impact at a demographic level. In this study we synthesize data from the literature and use it to produce a combined population dynamic and grazing impact model for *D. antillarum.* The aim of the modeling process is to gain a quantitative understanding of the recovery dynamics of this keystone species and inform the best avenues for future empirical studies and rebuilding strategies.

The model was explored to assess the impact of Allee effects and cultivation effects on the critical densities and sizes of urchins required for population persistence and population growth. The model was run under circumstances of both initial high macroalgal cover and low macroalgal cover to examine the impact of potential habitat modification.

Methods

We produced a combined population dynamics and grazing impact model for *D. antillarum.* Population dynamics are described by a size-structured, projection matrix model (Shepherd 1987; Lorenzen 1995) in which, the urchin population is divided into size classes such that the population density at time t is represented by a vector, n of densities-at-size. The equation that computes this vector for time $t + dt$ is:

$$n_{t+dt} = GS(n_t + r_t) \qquad (1)$$

Where G is a growth projection matrix, S is a survival matrix and r is a vector of new recruits obtained from the stock-recruitment relationship described below. Both G and S are dependent on population density and habitat state.

Growth

Due to conspecific competition for resources, *D. antillarum* exhibit density-dependent indeterminate growth, such that growth rates and asymptotic sizes decline with increasing population density. Growth in the model population is therefore described by a density-dependent extension of the von Bertalanffy growth function (VBGF) as described by Lorenzen (1996a) such that the diameter of urchin $D_{\infty}(B)$ is a linear function of biomass B:

$$D_{\infty}(B) = (D_{\infty L} - dB) \qquad (2)$$

Where $D_{\infty L}$ is the limiting asymptotic diameter in the absence of resource competition and d is the competition coefficient. This growth rate equation is used to produce the growth projection matrix, G by the method described by Shepherd 1987.

Mortality

The annual mortality rate, M in the model population is the sum of size-dependent baseline mortality excluding predation, M_b and additional predation mortality M_p.

$$M = M_b + M_p P_1 P_2 \qquad (3)$$

Baseline mortality, M_b is a function that describes the inverse relationship between individual size and mortality (Lorenzen 1996b). Predation mortality, M_p is a vector of values that describes additional size-dependent predation mortality. The values of M_p are estimated from a recent study that used a tethering experiment to show that predation risk was greatest in the smallest size classes and non-existent after a threshold size of 40mm (Clemente et al 2007) (Fig. 1).

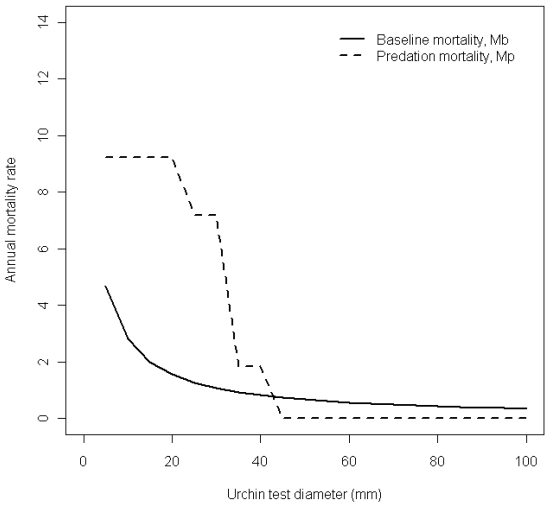

Figure 1: The shape of the relationships used to describe baseline mortality, Mb (Lorenzen curve) and predation mortality, Mp from Clemente et al 2007.

Due to the artificial nature of tethering experiments, the values assigned to M_p are considered to represent maximum levels of predation mortality.

Predation mortality is moderated by two parameters P_1 and P_2 which represent predation refuge effects associated with the hypotheses described previously, the adult spine canopy and the cultivation effect. P_1 is a function of adult surface area and acts such that predation risk, M_p declines as adult surface area and

thus spine canopy increases (Fig. 2a). P_2 is a function of macroalgal biomass and acts such that predation risk, M_p increases as macroalgal biomass increases (Fig. 2b). In this instance, macroalgal biomass is the variable used to represent the link between habitat cultivation and refuge availability.

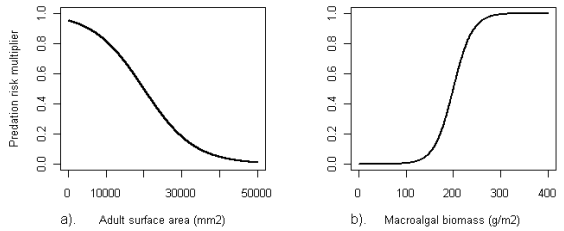

Figure 2: The shape of functions determining the extent to which predation mortality is applied to urchins. a). represents a decline in predation risk with increasing adult surface area due to the spine canopy refuge effect and b). represents the increase in predation risk with increasing macroalgal biomass due to the cultivation effect.

Incorporating an effect of macroalgal biomass required the inclusion of a simple biomass dynamic model for macroalgal growth and urchin grazing such that macroalgal biomass, A at time t is represented by:

$$A_t = A_{t-1} + vA_{t-1}(\frac{1 - A_{t-1}}{C}) - gU_{t-1}A_{t-1} \quad (4)$$

Where C represents the carrying capacity of macroalgal biomass, v the growth rate of macroalgae, and g, the proportion of algal biomass consumed per unit of urchin biomass, U.

Recruitment

Recruitment in the model population was described in two distinct ways; a stock-recruitment function relating to local adult biomass density and an externally driven density-independent level of recruitment. This distinction is important since speculation remains about the extent of larval dispersal in *D. antillarum* and whether larval supply to a local population is produced by that population or comes from source populations elsewhere (Bak 1985; Lessios 2001; Karlson and Levitan 1990).

The stock recruitment curve used to describe density-dependent recruitment was the product of two elements. The first was a depensatory curve representing an increase in fertilization success and larval settlement with increasing spawner biomass density (Fig. 3a). The second was a compensatory curve representing a decrease in larval survival at settlement as spawner biomass density continues to increase (Fig. 3b). Declining larval survival represents conspecific resource competition for suitable settlement space and acts to limit population growth to a carrying capacity.

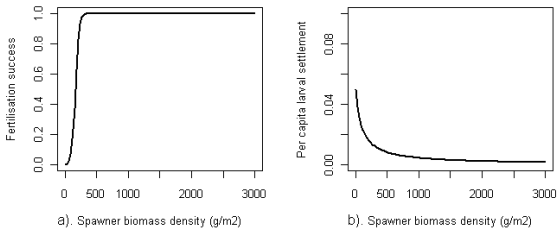

Figure 3: The two functions used to produce the stock-recruit relationship in the model population. A depensatory, logistic curve describing fertilization success (a) and a compensatory Beverton-Holt curve describing resource competition (b).

Biomass density was used in this relationship to allow for differences in gamete production between different sized urchins. The parameters of the curve were set such that they reflected maximum fertilization success beyond a threshold density of approximately one urchin per square meter (Levitan 1991; Levitan and Sewell 1992: Levitan and Peterson 1995) and created a maximum level of recruitment which reflects settlement numbers observed in the field prior to the mass mortality event (Bak, 1985). Figure 4 shows the resulting stock-recruitment curve (a) and the number of recruits produced (b).

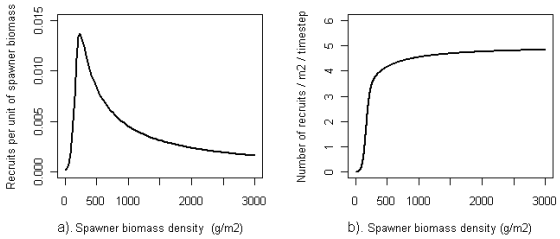

Figure 4: The stock-recruitment curve used to model recruits per unit of spawner biomass density (a) and the resulting level of recruitment per time step with increasing spawner biomass density (b).

Density-independent external larval supply was represented by the addition of a constant number of recruits at each time step.

Results

The *D. antillarum* population can persist in a low and a high abundance state. The low abundance equilibrium is associated with high macroalgal biomass whereas the high abundance equilibrium is associated with low macroalgal biomass. Due to the depensatory effects associated with adult spine canopy and macroalgal cover, a high initial abundance of large juvenile or adult *D. antillarum* is required for the system to switch to the high abundance state. (Fig. 5). Note that there can be a

substantial time lag associated with growth to high abundance equilibrium.

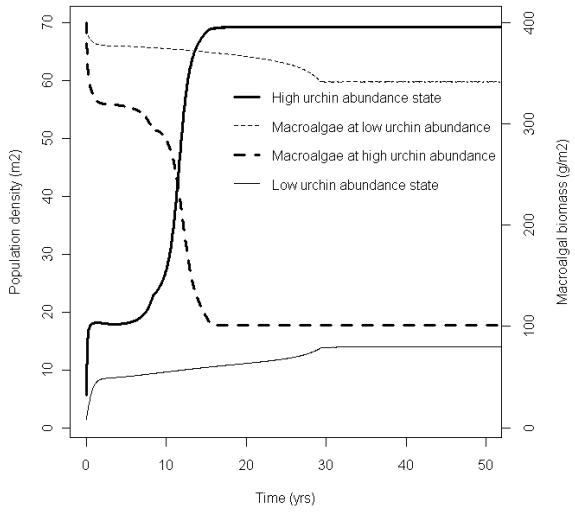

Figure 5: *D. antillarum* abundance and macroalgal biomass trajectories leading to low and high abundance equilibria, following releases of 1.4 and 5.6 medium-sized individuals per square meter. Solid lines represent urchin density, dashed lines represent macroalgal biomass and the thickness of the line denotes high or low abundance state with respect to urchins

We used the model to examine the number of urchins of different sizes required to kick-start rebuilding of a locally extinct population to a viable low or high abundance state, given high or low initial macroalgal biomass (Figure 6). Very high numbers of small urchins are required to establish a viable population, particularly when macroalgae are abundant. Much fewer large urchins would be required and establishment success with large urchins is less sensitive to the abundance of macroalgae.

The same method was used to assess threshold densities under a scenario of density-independent larval supply. The threshold densities for a high abundance state were very similar to those shown in Figure 6, the only significant difference being that there was no extinction threshold and the population would always persist even at low abundance.

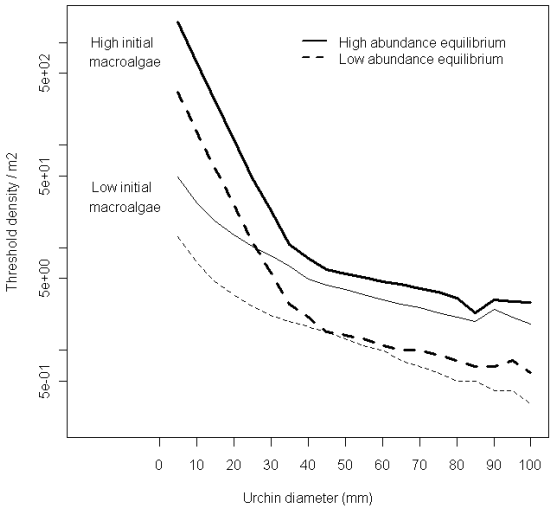

Figure 6: Threshold initial densities of urchins in each size class required to produce a population at low or high population abundance with an Allee effect in fertilization success, a spine canopy refuge and a habitat cultivation effect. Density thresholds are shown on a log scale.

Discussion

This study represents the first attempt to model the population dynamics of *D. antillarum* and gain a quantitative understanding of mechanisms impeding population recovery. The model represents mathematically key ecological processes and allows exploration of their population-level consequences.

The existence of both low and high abundance equilibria associated with high and low macroalgal biomass provides a possible explanation for the observed persistence of population densities much smaller than those seen prior to the mass mortality event of 1983. If, as our study suggests, macroalgal biomass plays a role in *D. antillarum* population dynamics then stable low population abundance may be due to the phase shift in macroalgae observed since the mass mortality event. However, the time lag involved in switching from the low to the high abundance state suggests that some low abundance natural populations may eventually recover

The existence of threshold densities for extinction and the two equilibria provides support for the potential for active rebuilding strategies. If we can aid populations to overcome threshold densities, then positive feedback associated with fertilization success, a spine canopy refuge and habitat cultivation may allow populations to persist and to recover to high abundance states.

As our studies show, even in the presence of sufficient larvae, population growth to high abundance is determined largely by juvenile predation mortality and the availability of predation refuges. The release of hatchery reared larvae alone is

therefore unlikely to prove effective if not coupled with some sort of refuge provision.

Model predictions show that urchin size and macroalgal biomass have significant effects on the thresholds required for population persistence and growth. In all situations, the larger the size of individuals, the lower the threshold densities required. It was also true that threshold densities were always lowest from a starting situation of low macroalgal biomass. These results suggest that adult release or translocation alongside prior macroalgal removal to mimic habitat cultivation may be the best rebuilding strategies. Adult urchins are less susceptible to predation mortality but also act to improve the survival of juveniles through spine canopy refuge and habitat cultivation. However, the large-scale release of urchins of large enough size at high enough density is logistically difficult since few are naturally available for translocation and we are yet to develop aquaculture technology for rearing adult *D. antillarum.* Previous translocation attempts have in fact proved unsuccessful due to low levels of retention and high levels of mortality (Miler et al 2007). A recent study in Curacao however, showed encouraging evidence that the provision of artificial predation refuges could greatly improve translocation success (Dame 2008). This supports the predictions of our model by highlighting the importance of predation mortality and refuge availability. An alternative to artificial refuge provision may be in the artificial removal of macroalgal to mimic the cultivation effect. Removal of macroalgae may free up existing predation refuges whilst simultaneously promoting larval settlement.

We propose that habitat modification in the form of macroalgal removal should be coupled with future attempts at translocation or hatchery release in order to promote survival and recruitment.

It is hoped that this study will promote further investigation into the role of macroalgal cover and habitat structure on larval settlement and juvenile survival in *D. antillarum.* This and future population dynamic models for *D. antillarum* provide an invaluable tool with which to quantify population recovery, direct future empirical work and advise rebuilding strategies.

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Measuring the effects of Marine Managed Areas: A global management effectiveness study

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Abstract. This paper presents preliminary results from a multidisciplinary research project on factors influencing the performance of Marine Managed Areas. This study is unique in that it investigates the impact of the timing of management interactions on a number of performance outcomes, across a global sample of MMAs. Data sources for the study comprise an extensive review of literature, key informant interviews, and household surveys in 24 villages from eight MMA sites in tropical regions. Analysis of household surveys indicate that adequate funds at the beginning, enforcement, shared benefits, community organizations, conflict management mechanism, and external support during implementation, and education and training programs, government support, and legislation today together account for 18% of the variance in the socio/ecological component of MMA performance. Alternative livelihoods, shared benefits, and leadership in the beginning, education and enforcement during implementation, and legislation, conflict management, and accountability today account for 17% of the variance in the empowerment and security component of MMA performance. Finally, the combined effects of community influence and education in the beginning, consultations, leadership and education programs during implementation, and influence, shared benefits, community organizations, and legislation today, account for 20% of the variance in the conflict component of MMA performance.

Key words: Marine Protected Areas, Economics of natural resources.

Introduction

The purpose of this study is to assess the factors related to the social and environmental effects of Marine Managed Areas (MMAs). In particular, it explores the links between socioeconomic, governance, and ecological circumstances, events, or interventions and changes in the human or natural environment of the MMA over time.

Attention to the links between MMA design and implementation processes and their longer-term outcomes has increased in recent years. In June of 2006, the Food and Agriculture Organization of the United Nations convened an expert group to develop recommendations for the use of Marine Protected Areas as a fisheries management tool. The panel, composed of social and environmental scientists from many disciplines, developed numerous recommendations for the effective design and management of MPAs. The panel noted that "the design of MPAs would benefit from more support for effectively designed and conducted studies of MPAs, emphasizing the diversity of situations in which MPAs have been applied, design and implementation processes, monitoring and performance, and ultimately, lessons learned." (FAO 2006)

This study support the goals articulated in the FAO document by investigating the relationships between socioeconomic, governance, and ecological factors and MPA outcomes. As noted by Pomeroy and Mascia in the FAO report, "The four principal elements of MPA design – decision-making arrangements, resource use rules, monitoring and enforcement systems, and conflict resolution mechanisms – directly and indirectly shape human resource use patterns and, ultimately, the biological and social performance of MPAs." (FAO 2006)

Material and Methods

Data sources for the study comprise an extensive review of literature, key informant interviews, and household surveys in 24 villages from eight MMA sites in tropical regions.

Figure 1 is a graphic representation of the analytic framework for this study. The outcomes (effects) of the MMAs are defined as a difference in the levels of MMA performance indicators from a time before the MMA was initiated to today. Socioeconomic, governance, and ecological factors were measured at three time periods – in the early phases of the MMA project, during implementation of the MMA, and today. The curved arrows in the graphic represent relationships between the socioeconomic, governance, and ecological factors that are related to the outcomes of MMAs. The dark, straight arrows represent

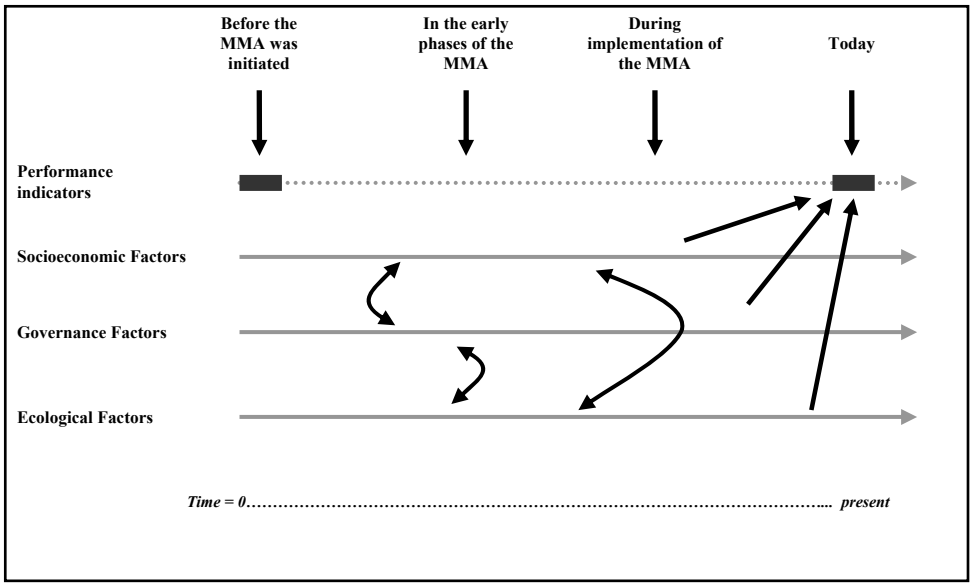

Figure 1: Analytic framework for GME study

relationships between independent variables (socioeconomic, governance, and ecological factors) and dependent variables (the change in performance indicators, or the effects of the MMA).

In order to conduct a quantitative assessment of the relationships between these dependent and independent variables, it was necessary to obtain comparable data from each MMA site. Although these sites were chosen based in part on the availability of secondary data, the data on socioeconomic and governance factors was not directly comparable across sites. Therefore, it was decided to conduct surveys with 40-60 households at each MMA. These surveys utilize the baseline-independent method for impact evaluation developed by Pomeroy et al. (1997) and widely employed by authors involved in coastal management research since that time (for example, Cinner et al. 2005; McClanahan et al. 2006). These household surveys provide comparable data on socioeconomic and governance factors present at each site and their timing, as well as the levels of socioeconomic and governance performance indicators before the MMA project and today. A significant difference in the reported level of a performance indicator is considered an effect of an MMA.

The household surveys were developed following a review of guidelines on the socioeconomic assessment of MMAs and coral reef management (Bunce et al. 2000; Pollnac 1998; Pomeroy et al. 2004). The survey instrument consists of three sections and about 50 questions. The first section contains questions about general household characteristics, respondent demographics, and community characteristics. The first section also asks the respondents whether they consider the MMA a success. The second section asks about the timing of factors that might be important in establishing and managing an MMA. These are our Critical Determining Factors, or CDFs.

The third section asks about respondent perceptions of the level of indicators (before the MMA and today) that could be considered the outcomes or outputs of MMAs. This study uses a visual, self-anchoring, ladder-like scale which allows for making fine ordinal judgments, places minimal demands on informant memory, and can be administered rapidly. Using this technique, the subject is shown a ladder-like diagram with 10 steps. The subject is told that the first step represents the worst possible situation. For example, with respect to coastal resources, the subject might be informed that the first step indicates an area with no fish or other resources, that the water is so foul nothing could live in it. The highest step could be described as rich, clean water, filled with fish and other resources. The subject would then be asked where the situation was before the MMA, and where it is today.

In all cases, local partners were enlisted to lead the implementation of the household surveys. The research team worked on-site with each local project leader to ensure that the survey questions were clear, and that they were appropriate to the communities being surveyed. Samples were drawn from the population of stakeholders in communities surrounding or near to the MMA who are involved with or are knowledgeable about the MMA. These individuals were either identified by the survey enumerators on-site by asking a screening question of randomly selected community members, or pre-

screened by MMA management prior to the research visit.

Results

For the overall sample, there was a statistically significant increase in perceived levels of all 12 of the impact indicators (p<0:01). In particular, there were large positive changes perceived in compliance, and ecological health and biodiversity. The next highest positive change was in livelihoods, with the smallest changes reported for peace and order and crime levels. Changes in the levels of effect indicators were not as pronounced for the subsample of fishers.

Indicator	T2-T1	P
Livelihoods	1.348	<0.01
Food security	.973	<0.01
Resource conflicts	1.054	<0.01
MPA conflicts	.933	<0.01
Participation	1.143	<0.01
Influence	1.233	<0.01
Peace and order	.877	<0.01
Crime	.771	<0.01
Village conflict	.935	<0.01
Compliance	2.247	<0.01
Ecological health	2.294	<0.01
Biodiversity	2.126	<0.01

Table 1: before/after MMA differences in perceptions of performance indicators

The next step was to reduce the number of independent variables for further analysis. Principal component analysis with varimax rotation was used to elucidate patterns of relationships between the degrees of change in the 12 indicators. The scree test was used to determine the number of components, resulting in three components, which account for a total of 62% of the variance in the data set.

	Component		
	1	2	3
Livelihoods	.639	-.065	.470
Food security	.659	-.038	.458
Resource conflicts	.254	.181	.773
MPA conflicts	.131	.244	.806
Participation	.032	.712	.310
Influence	.034	.682	.322
Peace and order	.340	.591	.073
Crime	.213	.686	-.053
Village conflict	.426	.358	.454
Compliance	.620	.384	.165
Ecological health	.794	.232	.109
Biodiversity	.841	.264	.079

Table 2: factor loading for principal component analysis

Items loading highest on the first component are clearly related to both livelihoods and ecology; thus, the component is named ''*Socio/ecological*.'' On the second component items related to participation and peace and order load highly, resulting in identifying the component as indicating ''*Empowerment and security*.'' Finally, items loading highest on the third component are related to various types of conflict in the community; hence, the component is named ''*Conflict*.''

We can then investigate the relative importance of the predictor variables in terms of their individual and combined ability to account for variance in the three indicator components. This can be accomplished with regression analyses, and most efficiently with stepwise regression analysis. The results of these analyses for the three components can be found in Table 3.

.

Independent variable	Stand. β coefficient	Prob.
A. Dependent variable: socio/ecological component score[a]		
ENF_IMPL	.166	.001
EDUC_TODAY	-.179	.000
SHAREBEN_IMPL	.165	.000
COMMORG_IMPL	.123	.010
GOVTSUP_TODAY	-.101	.027
LEGISL_TODAY	.127	.010
CONFLMGMT_IMPL	-.100	.032
EXTSUPP_IMPL	.171	.000
FUNDS_BEG	-.090	.050
B. Dependent variable: empowerment and security component score[b]		
EDUC_IMPL	.169	.000
LEGISL_TODAY	.097	.050
CONFLMGMT_TODAY	.126	.009
ENF_IMPL	.126	.006
ALTLIV_BEG	-.138	.003
SHAREBEN_BEG	.173	.001
LEAD_BEG	-.162	.002
ACCOUNT_TODAY	.111	.035
C. Dependent variable: conflict component scorec[c]		
COMMINFL_TODAY	.136	.011
SHAREBEN_TODAY	.162	.002
COMMORG_TODAY	.195	.000
LEGISL_TODAY	-.176	.000
COMMINFL_BEG	.145	.003
EDUC_BEG	-.143	.010
CONSULT_IMPL	.142	.008
LEAD_IMPL	.120	.020
EDUC_IMPL	-.121	.028

[a] $R = 0.450$, $R^2 = 0.202$, Adj. $R^2 = 0.186$, $F = 12.288$, $p<0.001$
[a] $R = 0.433$, $R^2 = 0.187$, Adj. $R^2 = 0.173$, $F = 12.603$, $p<0.001$
[a] $R = 0.469$, $R^2 = 0.220$, Adj. $R^2 = 0.204$, $F = 12.288$, $p<0.001$

Table 3: regression coefficients

The results in Table 3(A) indicate that adequate funds at the beginning, enforcement, shared benefits, community organizations, conflict management mechanism, and external support during imple mentation, and education and training programs, government support, and legislation today together account for 18% of the variance in the Socio/ecological component score.

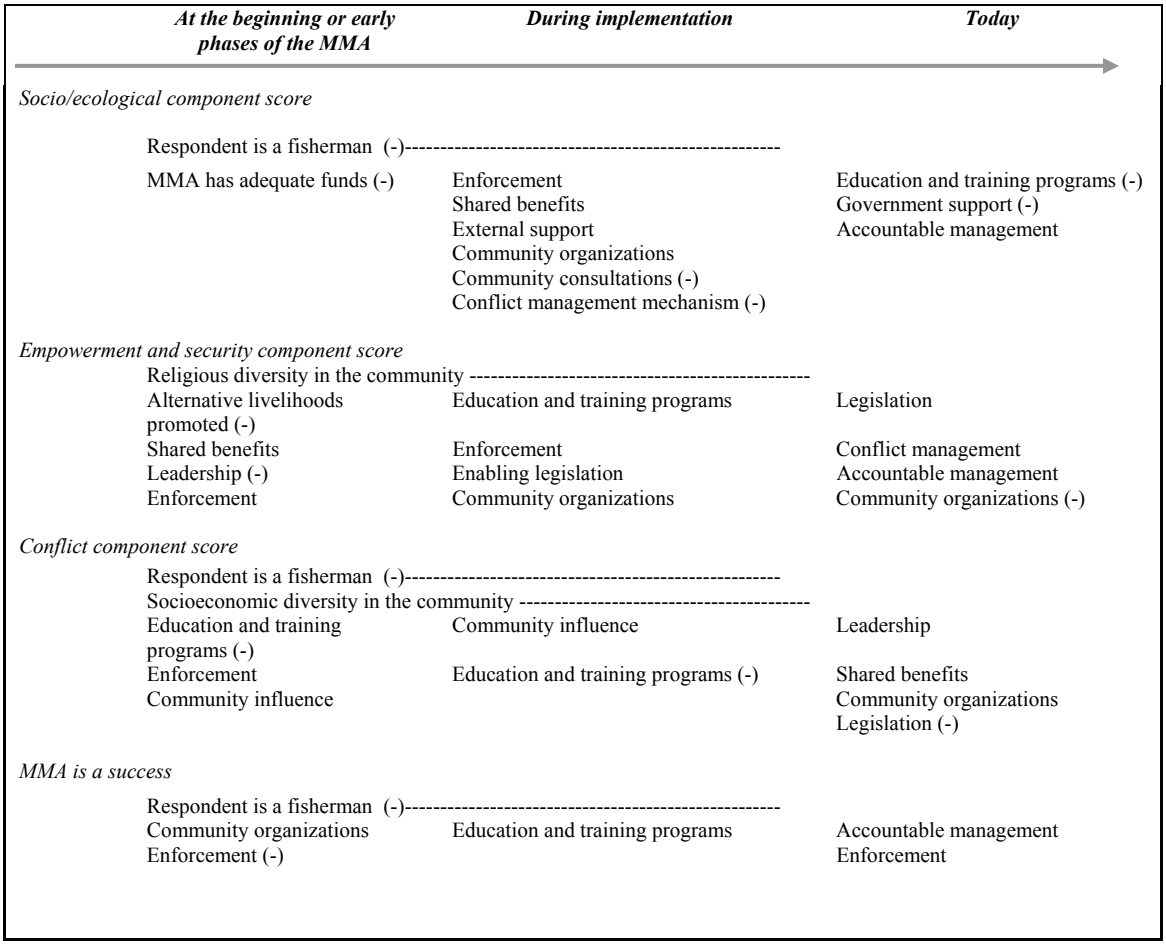

Figure 2: timeline representation of significant regressors

Likewise in Table 3(B), alternative livelihoods, shared benefits, and leadership in the beginning, education and enforcement during implementation, and legislation, conflict management, and accountability today account for 17% of the variance in the Empowerment and security component score. Finally in Table 3(C), the combined effects of community influence and education in the beginning, consultations, leadership and education programs during implementation, and influence, shared benefits, community organizations, and legislation today, account for 20% of the variance in the Conflict component score. Figure 2 is a timeline-type representation of these regression results.

Discussion

The most striking result of the component regressions is that some of the coefficients are negative, contrary to expectations. Recall that these are supposed to be critical *success* factors; therefore all coefficients would be expected to be positive. Thinking about the implications of measuring perceptions can help to make sense of these counterintuitive findings. For example, there may be some psychology at play in which respondents feel a sense of "bonding together in a difficult situation" that would explain why the coefficient on *adequate funds* is negative. Similarly, both *alternative livelihoods* and *leadership* at the beginnings are negatively related to the empowerment and security component score. Recall that the empowerment and security component is loaded most highly with perceptions of changes in influence and participation. It might be the case that if a strong leader comes in to a community with new ideas about changing traditional ways of life, that situation could be alienating and disempowering for some members of the community.

In general, the variables with negative coefficients are associated with situations that may be generated from outside the community itself – for example, strong leadership, legislation, education and training programs associated with the MMA, government support.

It is also noteworthy that some of the coefficients are positive for one component but negative for another. Education and training programs, for instance, have a positive effect on the *Empowerment and security*

component score but a negative effect on the other two components. Strong leadership has a negative effect on the *Empowerment and security* component score but a positive effect on the *Conflict* score. Investigating the robustness of this finding will be a fruitful area for further research.

This paper reports results from the first phase of an ongoing research project. Further steps will involve integrating quantitative ecological data from study sites into a comprehensive analysis. The authors welcome comments from readers to improve future versions of this report.

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Towards a close integration of social and natural sciences

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Abstract. Interdisciplinary teams are now commonplace in natural resource research centers. The evolution from pure biologists to a systems approach with ecologists and later on, social scientists and geographers reflects our understanding that no natural environment is exempt from human impacts; to protect the natural environment we must incorporate the human dimensions within the environment. Whilst conceding that interdisciplinary research is vital to the conservation of natural resources, I review our state of knowledge on interdisciplinary research and identify several barriers to successful integration. To address these, it is necessary to first articulate and understand these barriers. Only then can they be identified as they arise between researchers and successfully overcome in order to achieve sustainable natural resource management. This paper identifies four major barriers that hinder efforts at successful integration of social and natural science. These are (i) differences in epistemology, (ii) the place and hierarchy of each science within the wider community, (iii) researcher time constraints and (iv) publication pressures. The paper also provides a list of recommendations to overcome these issues in order to guide researchers through the challenges of producing integrated, quality, outcome driven research that serve both the community and the natural environment.

Key words: Interdisciplinary research natural social science

Introduction

A cursory glance at some of the major natural resource research centres around the globe reveal a trend towards interdisciplinary research teams. One example of this trend include the Australian Centre of Excellence for Coral Reef studies, which employs ecologists, biologists, geneticists, sociologists, economists and anthropologists.

The evolution of teams based in one discipline, e.g. biologists, to a system approach with ecologists and later the addition of social scientists and geographers reflect our understanding of the complexity of natural environment and its interconnectedness with human society and its impacts; we now realize that we must examine the system as a whole if we wish to protect it. For example, a relatively simple but pertinent question such as "How can the harmful environmental impacts of human activities be reduced?" can draw on the disciplines of demography, ecology economics, engineering, sociology, psychology, anthropology, political science, law and ethics in order to reach a satisfactory answer (Daily and Ehrlich 1999).

In addition, there is a recognition that, in order to successfully protect a natural resource such as coral reefs, there must be local support for the projects, and some incentive to preserve and manage the resource. Often in cases where no social scientist is immediately available, the responsibility falls to the natural scientist who has been working near or alongside the local community for many years to develop the incentive to preserve and manage the resource. However, as highlighted by the review of issues in interdisciplinary research that follows, an ad hoc, *cross*-disciplinary approach may not always be successful or achieve the desired outcomes; indeed authors such as Lele and Norgaard (2005) suggest that "charged with providing policy recommendations, natural scientists have to make judgments about how society works. They do not have adequate training to do this, but they are perhaps emboldened to do so by their positions and are likely to adopt simplistic models of social dynamics".

In this paper, I look first at the growing need for interdisciplinary research teams in natural resource management and environmental conservation. Next I draw on the literature, as well as personal experience, to identify and articulate some of the issues that such interdisciplinary research appears to face. Finally, and again based on existing literature in the area, some of the recommendations for overcoming these issues are discussed.

Literature review

When it comes to incorporating the social sciences into natural resource management, both natural scientists and social scientists may find themselves frustrated. Campbell recounts her experiences working with natural scientists at a turtle hatchery: "I was once asked to conduct research that would "show" that tourism was more valuable than an extractive-use project. Biologists may see results that do not see a desired conservation outcome as a

betrayal, both personally and professionally" (Campbell 2005). One reason for this, according to Campbell, is that natural scientists "may have unconscious or assumed expectations about what results of socio-economic studies will show", and which do not always reflect the research paradigms or priorities of the social sciences.

Indeed, some authors suggest that several misunderstandings may be at work here. First, a more palatable form of social science may be co-opted into a primarily natural science research team. In the words of Redclift (1998) "There is considerable evidence that some kinds of research in the social science can be fashioned to meet the demands of the natural sciences, e.g. demographic analyses can provide scenarios of food availability, surpluses, and resource scarcities. But is this social science? Is it rather what natural scientists think that social scientists are doing?" As a more extreme example we find the following "In my experience, natural scientists turn to social scientists to better package or market their eco/bio principles to the general public. True collaboration suggests mutual recognition of the distinct theory and methods that respective disciplines can bring to complex problems" (Fox et al. 2006).

Conversely, however, it has been argued that social scientists must also be able to "demonstrate that conservation-relevant social science is legitimate, worthy of pursuit and capable of answering questions of profound theoretical significance" (Mascia et al. 2003). Clearly there is some feeling amongst the natural scientists that social science has in the past failed to provide the conservation outcomes required for adequate protection of a natural resource, and a certain level of frustration felt by social scientists working within conservation-oriented research teams.

Aims and Objectives

The aims of this paper are to:

1. To articulate some of the issues that face interdisciplinary teams
2. To help think about them and identify them as they arise
3. To make some recommendations for overcoming them

Material and Methods

The principle methodology for this paper is a review of the literature on interdisciplinary research, with a particular focus on those issues that arise in mixed social and natural science teams. The review is set within the author's own experience as a graduate in marine and environmental sciences, a doctoral degree in tourism, and a postdoctoral position in the School of Business, managing a research project on sustainable use of marine resources within a tourism context.

Results

Based on some of the ideas presented above, as well as a more complete review of the existing literature, it would appear that there are four main issues that may lead to communication breakdowns in interdisciplinary teams. These are (i) differences in epistemology, (ii) the place and hierarchy of each science within the wider community, (iii) researcher time constraints and (iv) publication pressures.

(i) Differences in Epistemology:

Differences in epistemology is arguably the biggest area of potential conflict between social and natural scientists. (Lele and Norgaard 2005). It can be suggested that natural scientists believe in an absolute truth, which should be reached through a reductionist, positivist approach to reach general consensus, whilst social scientists may employ far more interpretive techniques that encourage debate and move away from consensus, admitting the existence of competing controversial universalities or distinct epistemic communities (Redclift 1998).

A range of other related issues also appear in the literature. For instance, language issues may arise, as differences between a clearly technical language and a science built upon more common language may lead to misunderstandings. In particular social scientists who often use common language in their research complain that the uninitiated reader, may mistakenly conclude that he understands what is being said (Pearce 2008, pers. comm.; Wear 1999). An example from personal experience is the confusion between the terms tourists and holiday-makers. In these cases, interdisciplinary research requires a clearer description of framing theory and methodology, and some insights into constitutive metaphors in order to build a common language that may usefully serve both groups of researchers (Wear 1999).

A second sub-issue resolves around the sensitive topic of values. This issue arises at all stages – choice of questions, theoretical positions, variables chosen, styles of research. And whilst natural scientists like to think that they are value-neutral, e.g. through the use of the passive tense in reports, social scientists recognize the importance of the context of all research (Lele and Norgard 2005) allowing subjectivity to become part of the research. Moreover, Campbell (2005) points out that advocacy issues can be strong in conservation biology, sometimes to the detriment of science. The goal of conservation biologists is to preserve biodiversity. There is often therefore a value placed on social science that is able to achieve this conservation goal more effectively through

deliverables. Campbell suggests however, that issues of advocacy will influence human subjects through their perceptions of the overall research team and project and not just by the social scientists with whom their interact. This will have detrimental effects on the quality and validity of the social science.

(ii) The place of science in society:
Another sensitive issue that is identified in the literature is that of the place of science in society and associated issues of funding (Roughley 2005). A form of hierarchy of intellectual rigor appears to exist in the sciences from the so-called "hard" sciences such as physics and chemistry through to "softer" sciences such as ecology and psychology, leading finally to anthropology and sociology. This hierarchy of worth, and the funding discrepancies associated with it has been accused of creating arrogance or defensiveness within disciplines, and forming a barrier to successful interdisciplinary research. Some authors therefore argue for personal characteristics of interdisciplinary researchers to be carefully considered, and point to the need for trust, patience, responsibility and honesty (Naiman 1999).

This may also help to overcome apparent issues of power imbalances, whereby research leaders are often based in the natural sciences and the natural scientists, by the nature of research methods, may outnumber the social scientists. The interpersonal skills of the research leader will therefore also play an important role in creating good interdisciplinary science within large teams. He or she will need to ensure that everyone shares the same general vision, but have specific parts of the overall project so that they have ownership. This may require the commitment of senior people who have little to risk professionally and are anxious to involve bright junior people in their efforts. Furthermore, whilst there is often only one social scientist in the team, this person will be required to represent a broad suite of social science concerns and represent the team in a social context, e.g. educate local people or fix socio-economic problems. Both of these roles are demanding and can be exhausting, and again, require good interpersonal skills and a strong commitment to the project.

(iii) Issues of time:
When talking about interdisciplinary research, it has been suggested that mutual understanding and cooperation build slowly (Daily and Ehrlich 1999). Social interaction and long-term association that allow friendships to develop create stronger interdisciplinary teams. Furthermore, it takes time to develop common language and familiarise oneself with other disciplines, as well as build the research program around several disciplines so that it may be truly inclusive, empowering and truly reflect the issues at hand (Naiman 1999). On the other hand, conservation has a certain level of urgency associated with it. It may be therefore that much of social science research and methodology are a luxury that conservation practioners cannot afford (Brosius 2006).

Another issue that is related to time constraints is the issue of good science: the commitment of time and energy into understanding other disciplines invariably detracts from the time and commitment put into maximising one's own mastery of a single discipline. The result is a perception that interdisciplinary scientists are less competent or accomplished, and that interdisciplinary science is less exacting (Fox et al. 2006).

(iv) Publishing Issues
A final major issue that has been noted in the literature comes with the publication process for researchers. Pressures to publish in high ranking academic journal may limit incentives to publish in the newer interdisciplinary journals, or in journals that may not lead to high citation rates. Furthermore, reviewing processes might be more difficult as interdisciplinary articles require more time and effort from editors and reviewers. Review processes for interdisciplinary journal are also felt to be biased towards natural scientists, and reviewers are often interested in and familiar with the issues addressed but unfamiliar with theories and methods. According to Campbell (2005) this does not stop reviewers from suggesting related revisions, usually inappropriately. It is suggested that there is a need to expand list of social scientists on editorial boards and use these more fully.

Discussion
Whilst this review does not provide a comprehensive list of all issues facing indisciplinary research teams, it had highlighted some of the key issues. By articulating some of the more commonly cited issues that arise in interdisciplinary research, it is hoped that researchers in this situation will be able to recognize and circumvent barriers as they arise. Some recommendations highlighted in the literature, particularly in Naiman's (1999) and Mascia *et al.*'s (2003) papers, include the following:

- Make a conscious commitment to cooperate, and visualise and acknowledge the personal and professional sacrifices as well as the rewards
- Cooperate with colleagues who have a similar level of commitment to team research. Team research is freely sharing ideas, a commitment to excellence, being honest and having an arena of mutual respect in which to work

- Choose people that are willing to assume and share leadership and responsibility.
- Take the time to educate new team members and to ensure their ownership of a significant aspect of the project. Build confidence as well as ownership
- Find ways to encourage continuous communication of new ideas, even though one may not always agree, do validate the person as well as his/her willingness to share
- Never forget that we are all individuals with our own strengths and weaknesses. Value everyone in the group, not just those with the best work habits or ideas.
- Learn how to continue learning in an ever changing world, practice tact and patience; demonstrate respect.
- Encourage social scientists to make a greater effort to initiate and obtain funding for their own environmental management or conservation projects, to which they could include natural scientists.
- Hire social scientists for leadership positions and provide them with the mandate to build social science into organisational decision-making
- Enlist social scientists to develop and manage "rapid social assessment" programs, which would provide decision-makers with a rough sketch of critical social information at potential conservation sites through short-term but intensive inquiry.
- Document and share success stories that illustrate the value of social scientific information to "on the ground" conservation results. Such success stories not only foster organisational learning, internal support and conservation success, but also justify donor and organisational investment in the social sciences.

By adopting some or all of the recommendations outlined above, it may be possible to achieve a greater integration of natural and social sciences and achieve significant progress in interdisciplinary research. In conclusion, the greater integration of social and natural science may adopt a more outcome driven approach that creates good, creative interdisciplinary research, in turn allowing communities to adapt to rapidly changing natural environments. Finally, more dialogue between social and natural scientist is encouraged through such media as peer-review publications (examples include the Journal of Environmental Management, Natural Resources Journal and Society and Natural Resources) and conference presentations. The emerging interdisciplinary teams can and should add new perspectives to each science and allow them to learn from each other, particularly, in the words of Lele and Norgaard (2005), once each side acknowledges what the other does not know.

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The political aspects of resilience

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Abstract. This study investigates the political aspects of resilience in social-ecological systems. Based on long-term anthropological fieldwork in the Calamianes Islands, Philippines, the study focuses on the diverse and contested human interests that make up social-ecological systems. In the Calamianes, what promotes the interests of one group of people may impact negatively for another group of people, or the ecosystem in which they live. Fishers, for example, have struggled greatly to preserve their patterns of marine resource use, and to oppose various forms of regulation that have been introduced. Following Armitage and Johnson (2006), this study has found that deciding 'for what and for whom are we trying to promote resilience?' becomes a critical question. Answering such a question will require decisions that will favour certain elements or resource users within any social-ecological system, and disadvantage others. The study concludes therefore that such political aspects of resilience thinking require greater attention, and that more attention could be paid to negotiations over tradeoffs among various stakeholders, if the resilience concept is to be more widely accepted in policy and management arenas.

Key Words: resilience, social-ecological systems, politics, anthropology, Philippines

Introduction

This paper emphasises how greater attention could be paid to the political nature of resilience, and of policies designed to promote resilience. Building on the notion that social-ecological systems are made up of diverse and contested human interests, the paper shows how any attempt to promote resilience for the overall social-ecological system will have differentiated impacts and effects among the diverse elements within it. The paper will show how policies designed to improve social-ecological resilience were transformed, and ultimately rejected, by fishing communities concerned with resisting these policies. Here, there is a significant gap between the particular social and economic interests of fishers, and the resilience of the broader social-ecological system. The paper suggests that resilience must therefore be understood within its political context, and that we need to pay more attention to the need for negotiations over tradeoffs among various stakeholders.

Methods

The paper takes an anthropological perspective to examine the issues surrounding the political aspects of resilience and social-ecological systems. Research was conducted for twelve months between September 2005 and January 2007 in the Calamianes Islands. The author was based in two locations during this period: Esperanza, a small coastal community within Coron municipality, and Coron town, the capital of Coron municipality and the largest town in the Calamianes. Coron municipality was chosen to study the political aspects of marine resource management because of the high importance of commercial and small-scale fishing, and the high number of marine resource regulations implemented at the time of fieldwork. Esperanza has a particularly high population of fishers, and several of the MPAs that are discussed in the paper were located in fishing grounds used by fishers from Esperanza. Specific methods adopted for the study included observation at meetings, and numerous formal and informal interviews with a variety of different stakeholders. These included dive operators and divers, fishers of all ages and types, government officials, and non-government organization workers. Interviews with fishers were conducted in Tagalog. In addition to the in-depth data gathered from Coron and Esperanza, the author frequently visited and interviewed residents at other locations of the Calamianes.

Diverse and contested human interests

Scholars in resilience theory have typically tended to reduce the differences between social and ecological systems. Berkes et al. (2003), for example, state that 'the delineation between social and natural systems is artificial and arbitrary'. Social science scholars, however, have tended to emphasise the distinctiveness of human systems because of the vital presence of human agency. Social scientists have long affirmed that human relations with the environment cannot be reduced to energy flows; they contain value and agency (Burnham and

Ellen 1979). Because of the complexity of human agency, this means that social systems contain diverse and contested human interests. It follows that resilience has to take account of such contested interests if it is to be practically applied. As Johnson and Armitage (2006) point out, '[f]or resilience to be useful in assessing social institutions and in marking roads to sustainability and social justice, it has to be situated in the context of complex, contested and changing human interests, and the uncertainty of the outcomes of human interactions'.

The Calamianes Islands can be seen as an example of a social-ecological system that contains diverse and contested human interests with regard to the management of marine resources. The main livelihood for the region is a variety of fisheries, both small-scale and commercial. While several fisheries have boomed and busted since the 1970s, the live fish for food trade is currently dominant; an estimate of the value of the trade in the Calamianes for 2002 was about US$5.3 million (Pomeroy et al. 2005). This fishery has been extremely profitable for local traders and fishers, but has also been highly ecologically destructive, characterised by overfishing and the common use of sodium cyanide (Fabinyi 2007). Stakeholders from the fishing industry have generally attempted to preserve the status quo, with minimal or no regulation of their activities.

Conservationist organisations have been promoting the development of marine protected areas to work with dive tourism, and regulations designed to reform the live fish trade, which included a lengthy closed season. Much government planning and the everyday lives of residents in the broader region can be characterised by contestation over how best to access, exploit and manage these marine resources (Eder and Fernandez 1996).

Socio-political interests vs. social-ecological resilience

While not explicitly framed in terms of resilience theory, both sets of environmental reforms were attempts to promote social-ecological resilience by reducing both the long-term poverty of the coastal communities of the Calamianes, and degradation of the marine environment. The policy brief behind the development of the live fish regulations, for example, stated that '[t]he policy goal is for a sustainable fishing industry in Palawan Province that ensures viable fish stocks, ecosystems and livelihoods for present and future generations' (Pomeroy et al. 2005). Similarly, marine protected areas have been cited as a means of promoting resilience. In the Calamianes, their development was aimed at both maintaining the ecological integrity of particular marine ecosystems, and at livelihoods through the development of tourism and by increasing the overall level of fish stocks.

MPAs

The ways local fishers understood and responded to many of these MPAs was extremely significant in determining their ultimate outcomes (Fabinyi 2008). Importantly, fishers placed their fishing practices within a social and political context. Small-scale fishers generally represented their patterns of fishing as possessing two key features: it was harmless to the environment, and it was closely tied to poverty. In contrast, tourists and tourist businesses were frequently objects of resentment by fishers, the latter seen as undeservingly profiting from the beautiful reefs of the Calamianes. From this perspective, any regulations to try and reduce problems of environmental degradation should not impact on the small time fishers, who could not afford it. They felt that any regulation that interfered with the activities of small-scale fishers, such as MPAs, would have to be accompanied by financial benefits of tourism. These perceptions meant that some [particularly younger] fishers were sometimes opposed to the creation of MPAs if they were not seen as benefiting local communities, and indeed felt justified in still fishing within them. Fishers would only tend to support MPAs if they did not impact significantly on their fishing practices, focusing instead on taxing tourists. Essentially, the perspective of many local fishers was that MPAs which interfered with existing fishing activities were to be opposed, unless they gave significant benefits in terms of tourism money.

The planning processes for many of these MPAs illustrated this perspective of local fishers clearly. What was notable was the ways in which coastal people refused to allow the MPA to have any impact on their particular patterns of marine resource use. During the planning meetings for example, the core or 'no-take' zones were changed from the original locations drawn up by the marine scientists in the conservation projects to account for the presence of several fish traps owned by local residents. And, in the buffer zones of the MPAs, residents ensured that their fishing practices would be allowed, while actively pushing for the fishing practices of neighbouring communities to be disallowed. For all of the fishers, the MPA had no ecological focus, but was viewed as something to support the community; something that was solely about assisting and protecting their livelihoods.

Another key aspect of this planning process of the MPAs could be seen in the desire among fishers to obtain greater benefits out of the tourism industry in the form of user fees, which

were demanded by participants as an essential component of the MPA. For many coastal residents in the Calamianes, the socio-economic differences between foreign tourists and themselves were stark and inescapable. Foreign tourists were typical of the 'rich foreigner'. Their very presence in a place like the Calamianes, pursuing nothing but leisure activities like SCUBA diving, was proof to locals that they had incredible amounts of money to burn. Versions of refrains such as 'You foreigners throw money away like it is rubbish' were commonly stated assumptions among local fishers. In contrast, the Philippines was understood to be the most poverty-stricken place on Earth.

The development of MPAs in the Calamianes can be seen as stimulating a sense of territoriality over the protected areas. In effect, they produced an artificial form of marine tenure or ownership among coastal communities. The rationale behind fishers' support for these MPAs had less to do with conservation or even fisheries management, but more to do with an expression of political, livelihood claims. Fishers viewed MPAs as a way in which they could address some of the massive inequalities between themselves and those involved in the tourism sector. The claims of fishers about MPAs were based not on a logic of environmental management, rather on a belief that their poverty ought to be the focus of any external environmental intervention. For fishers, no purely technical, scientific management of MPAs would be fair or legitimate without taking into account their claims. As it turned out, these claims favoured the development of particular types of MPAs that had minimal restrictions on local fishers, were loosely enforced when it came to fishers, and involved user fees for tourists.

The status of many of the MPAs in the Calamianes could be seen as somewhat ambiguous. Many were being created, but most of them were marked by serious shortcomings and limitations. Core zones were often minimised as much as possible and seen as a concession to conservationists, buffer zones were adapted to include the fishing techniques and gears of local fishers, and enforcement was rarely effective when it was conducted by locals. Whether the MPAs produce the increase in fish stocks as desired by the conservationists (and the fishers) remains very unclear because of these sorts of shortcomings. So, from this perspective, the proliferation of MPAs is not so much a victory for wise fisheries management or the promotion of social-ecological resilience, but more a way in which local fishers and some sympathetic local government officials were able to successfully advance their interests.

Live Fish Trade Regulations

The second piece of environmental management that coastal communities responded to was the implementation of a series of regulations designed to reform the live fish trade. These regulations were approved in March 2006 at the provincial level, but the political system of the Philippines requires that provincial laws have to be approved by municipal laws before they are actually implemented in those specific municipalities. So, for most of 2006, the provincial council and the various municipal councils that supported live fishing were locked in a stalemate arguing about the new live fish regulations.

At different points through 2006, the provincial government threatened the municipalities involved in live reef fishing with a moratorium on the renewal of all live reef fishing accreditations if these regulations were not adopted. Finally, in December, after a whole year had passed without the municipal governments creating specific municipal ordinances, the provincial Bureau of Fisheries and Aquatic Resources enforced a ban on all live fish exports from Palawan. Immediately after this, furious lobbying and negotiations occurred. Fishers went to the traders that supported and financed their fishing activities, asking for help. They argued that the imposition of the regulations was extremely unfair; that if there were any problems in the live fish trade it was, according to them, only because of a small number of fishers who used cyanide. The rest of the fishers who used legal methods should not be punished for the actions of a few. They argued that by imposing a closed season and creating MPAs in the best fishing grounds, this would send them back to the poverty-stricken lives they had lived before the introduction of the live fish trade in the Calamianes. In particular, fishers emphasised that the live fish trade was the only significant commercial industry in the Calamianes, and no alternative livelihoods existed that could even come close to the live fish trade in terms of financial benefits. Many people would go hungry, the fishers argued, and other community members also protested at the regulations, claiming businesses such as general stores would also suffer. Gaining national headlines in the newspapers, more than 500 fishers eventually trooped to the capital of the province to protest at the creation of the regulations.

It seemed for a while that the provincial council was not going to back down, and that the moratorium would hold. Eventually however, they gave in to intense pressure by their local constituents. The ban was overturned at the New Year, and shipments were allowed to go through again. Another year and a bit on in 2008, and the

live fish trade continues in Coron with little regulation.

Adger has defined social resilience as 'the ability of communities to withstand external shocks to their social infrastructure' (2000: 361). In the terms of this and other definitions the overall social resilience of fishers was probably quite low, given their high dependence on fishing and their lack of economic flexibility. However, suddenly taking a large part of this livelihood away through powerful regulations would have certainly increased their vulnerability in the short-term at least, no matter how immensely important the ecological justifications were. In both of these cases, fishers were acting against what they perceived as a clear potential shock to their social and economic infrastructures. In a region with few available alternative livelihoods, the introduction of MPAs and a closed season for the live fish trade would have had a significant economic impact. It was therefore in their interests to oppose the live fish regulations and to influence the implementation of MPAs the way they did.

Discussion

In this context then, a contradiction can be observed between the resilience of the overall social-ecological system, and the interests of fishers and local government. Supported by the municipal government, fishers were able to promote their interests by manipulating the design of MPAs so that their social and economic benefits remained, and by rejecting the live fish trade regulations that would have heavily disrupted their most important economic livelihood. In doing so, such outcomes compromised the ecological resilience of the social-ecological system. This basic contradiction leads to a fundamentally important question about social-ecological resilience — how is it possible to promote social-ecological resilience when any social-ecological system is composed of diverse and contested human interests? Or as Armitage and Johnson ask, '[h]ow do we balance these divergent interests and the interest of ecological sustainability to define the ideal resilient system?' (2006).

Many analysts have argued strongly for the principles of 'good governance', as articulated through the themes of adaptive co-management, polycentric governance, interactive governance and other related terms (Armitage 2008). Lebel et al. (2006), for example, hypothesise that the existence of participation, polycentric and multilayered institutions, and accountable and just authorities can be associated with an increase in resilience for social-ecological systems.

But while in many cases good governance may clearly be associated with resilient social-ecological systems, in many cases it is not, as Lebel et al. also acknowledge (2006). As Armitage (2008) argues, governance attributes such as those described by Lebel et al. (2006) are 'productive and important, but they are circumscribed by context, and… provide only partial direction for governance innovation. They represent a set of "prescribed" and normative governance values or principles'. During the introduction of the environmental regulations in the Calamianes, for example, attention was paid to various principles of good governance outlined earlier. Indeed, it was the very existence of strong participation by the fishers in the design of the MPAs that led to their ecological compromise. In both instances, governments ultimately were accountable to their constituents and responded to what the majority of the stakeholders wished for, which were MPAs that fulfilled short-term social and economic objectives but had little ecological function, and the rejection of regulations for the live fish trade that would have been beneficial ecologically, but undoubtedly socially and economically damaging in the short-term. This can be seen as an example of governance that is good when understood from the perspective of the interests of fishers and the municipal government, but clearly damaging when viewed from the long-term perspective of the social-ecological system.

Some have lauded the potential of good governance to provide a situation which will be mutually beneficial to all stakeholders. In contrast, perhaps what we need to be more aware of is the inevitability of 'hard choices' in marine resource management (Bailey and Jentoft 1990). Any decisions taken at the scale of the social-ecological system are going to privilege some elements within this system, and alienate others. As in the case of the Calamianes, what may promote resilience for the system as a whole may not always be in the direct interests of particular groups within that system. Therefore, resilience, and activities undertaken to promote resilience at the scale of the social-ecological system, must be seen as a political concept. Resilience, like any other concept for ecological management, including those that emphasise good governance, cannot remove the need for political decisions and negotiations among diverse stakeholders to be made in particular local contexts.

Similarly, practitioners in the field of integrated conservation and development projects have recently emphasized the need for greater attention to and understanding of the goals and interests of all the stakeholders around protected areas, noting that win-win solutions are usually unachievable: 'Once these different interests are identified, clarified, and understood,' they argue,

'the opportunities for negotiation and tradeoffs can be explored' (Wells et al. 2004).

This paper has argued that we need to place greater emphasis on such political aspects of resilience if it is to succeed as a viable and practical management or policy concept. While resilience may be a useful metaphor to understand features of social-ecological systems, focusing on issues at this level of abstraction actually obscures somewhat the diverse social and political relations that actually make up these social-ecological systems, and how resilience affects and is affected by these relations in different ways.

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New eco-development initiatives involving local people in the conservation of Mahatma Gandhi Marine National Park, Wandoor (India)

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Abstract. Mahatma Gandhi Marine National Park situated at Wandoor about 29 km from Port Blair is among the first three Marine National Parks in India. Established in 1983 with the objective of conserving the unique marine diversity and the coral reefs, it also comprises 15 uninhabited islands. There are 6 villages in the adjoining area with a population of about 4157. The main occupation is fishing. No fishing is allowed within the Marine National Park but routes are demarcated for providing safe passage to local fishermen. MGMNP is an important eco-tourist site because of its unique marine biodiversity. In order to involve the local people in the conservation efforts, the Park authorities initiated action at a small level in 2003-04. The tsunami of 2004 affected not only tourism but also the efforts of Park authorities in people's participation. Increased tourist inflow in the last two years led the park authorities to revive their eco-development initiatives. New initiatives are being taken for providing livelihood means to locals and involving them in the conservation efforts.

Key Words: Eco-development, tourism, fishing routes, livelihood options, Peoples' participation

Introduction

Mahatma Gandhi Marine National Park (MGMNP) was established in 1983 under the Wildlife (Protection) Act, 1972, with a view to protect and conserve the rich marine life found in this area. This was the first Protected Area notified in this Union Territory and also among the first three Marine Protected Areas of India. It is situated around 29 km from Port Blair, the Capital town of the Andaman & Nicobar Islands. The otal area of MGMNP is 281.50 km^2 which includes 220.5 km^2 of territorial water and 61 km^2 of land area of 15 Islands located within the boundary of the National Park. All the Islands within MGMNP are uninhabited. Only two Islands namely Jollybuoy and Redskin are open for tourism, but no night halt is allowed. The Park is completely free from all rights. It is managed by the Department of Environment & Forests, Andaman & Nicobar Administration.

Biodiversity Status

The Park is known for its rich biodiversity-both plant and animals. There are 279 species of plants. Most of the islands have tropical evergreen forests and mangroves on the fringes. The tree species include *Dipterocarpus, Terminalia, Pterocarpus, Diospyrus marmorata, Artocarpus chaplasha* etc. Littoral species are dominated by *Manilkara littoralis* and important mangrove species are *Rhizophora, Bruguiera, Avicennia officinalis, Ceriops tagal.*

The terrestrial fauna include Andaman wild pig, spotted deer, water monitor lizard and a variety of snakes including sea snakes. The birds include Andaman teal, white-bellied sea eagle, parakeets, sea herons among many others.

The Park is rich in marine biodiversity. More than 280 species of fish, 57 species of mollusc, 52 species of echinoderms, 122 species of corals belonging to 54 genera, salt water crocodiles, four species of sea turtle, dolphin, dugong, a variety of sea weeds and sea grasses are reported to occur (Kulkarni et al., 2000, 2004).

Socio-economic status of communities living around

There are no inhabitants within the boundary of MGMNP. However, six villages adjoin the boundary of the Marine National Park. Population figures of these villages as per 2001 census are given in Table 1. Occupational pattern of the villagers is depicted in Figure 1 (Kulkarni et al., 2004). The data shown in Figure 1 is pre-tsunami data (i.e., before 2004). There has been a significant

Table 1: Population of villages adjoining villages

Sl. No.	Name of village	Population
1.	Wandoor	1511
2	Hashmatabad	616
3	Guptawara	716
4	Manglutan	575
5	Manjeri	585
6	Rutland	154

change in the occupational pattern after the area was struck by a Tsunami in December 2004. A number of farmers lost their land due to submergence and have turned to other opportunities. A number of fishermen have taken up tourism related activities.

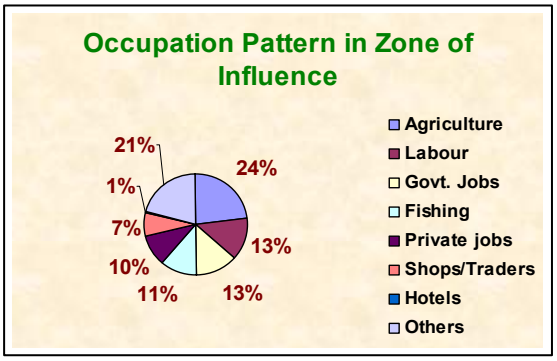

Figure 1: Occupational patterns of locals near MGMNP Source: Kulkarni et al., 2004

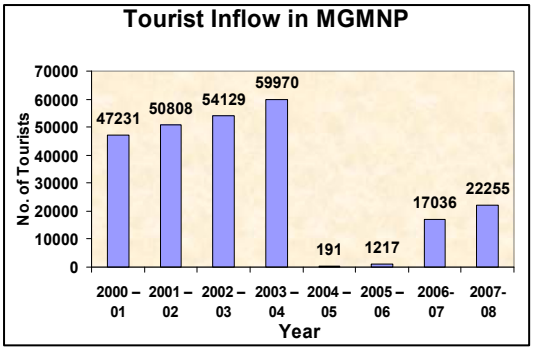

Fig. 2: Tourist inflow to MGMNP.

This Park is an important tourist destination. The inflow of tourists was adversely affected by the Tsunami but in subsequent years the inflow increased again (Figure 2). Though the number of tourists visiting the Marine National Park appears to be still less than 50% of visitors in 2003-04, but the number of tourists visiting Wandoor in 2007-08 was higher than in 2004-05. Two new tourist attractions (New Wandoor beach and Mahua Dera beach) were identified and developed by the local people. Even without proper record, increase in the number of tourists was confirmed by the local people and the Park authorities.

Objective

The objective of the present study was to evaluate measures taken by the Park authorities to address the expectations of the local communities residing in the vicinity of the Park and to involve them in the conservation efforts being taken up in the MGMNP, Wandoor.

Methodology

Four villages (Wandoor, Guptawara, Hashmatabad and Manglutan) fell in the zone of influence. In addition to collection of basic data on the socio-economic profile of these villages, a survey was conducted to understand the extent of

- Awareness about the MNP
- Its objective and impact on occupations
- Their perception about changes taking place
- Their expectations
- Relation with Park Authorities
- Initiatives by the Park Authorities

A questionnaire was developed and interviews were the main tool of data-collection. Stratified random sampling design was used. Sample size of each stratum was based on population as well as the relative importance. Strata-wise percent distribution of sample size is depicted in Figure 3.

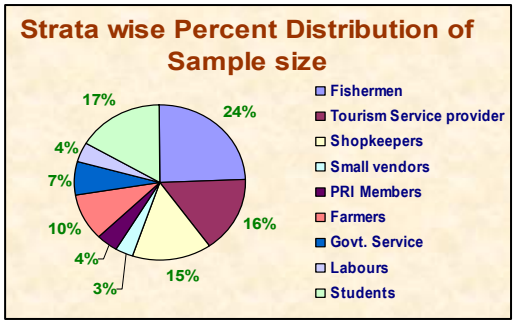

Figure 3: Occupations in the zone of influence of MGMNP.

Results and Discussion

It is revealed from Table 2 that there has been anincrease in economic activities particularly in the post-tsunami period. The number of bus services from Port Blair to Wandoor has almost doubled. The number of private taxis visiting Wandoor per day is presently around 50. The numbers of lodges

and restaurants did not increase much, probably because tourists visit this area during day time for enjoying the beach, swimming, snorkeling and viewing marine life. Local tourist services have also increased from nil to 12. It is quite evident from this Table that local people have realized the potential of tourism as an important livelihood option and therefore not only have they identified new areas of tourist attractions but have also acquired loans from Bank mainly to purchase boats for tourism purpose.

Activity	Pre 2000	2000 -04	2004 -08
Restaurants & Lodges	3	5	6
Ration shops	1	2	3
Bus Service (No. of trips)	16	16	28
Schools	2	2	2
Tourist Services	Nil	Nil	21
Other commercial Establishments	7	8	12
Loans availed for boat	Nil	Nil	37
New tourist Areas identified by local people	Nil	Nil	2

Table 2: Pattern of emerging economic activities

Other important findings of the survey are as follows:

- About 93 % of the local people were aware of the objectives of the establishment of MGMNP in some form or the other. Of the total people interviewed, 66 % knew that the Park was constituted for protection of marine life, 22 % think that the purpose was for promoting tourism and 5% think it to be protection of Wildlife. (Figure 4).

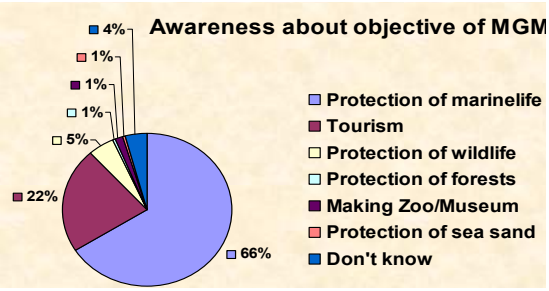

Figure 4: Awareness of the MGMNP.

- On effect of establishment of MGMNP on their occupation, 38 % responded that it has adversely affected their occupations. Of this 91 % were fishermen as fishing is not permitted inside the Park and they have to go to far distances for fishing. Thirty six percent responded that the establishment of the Park has favorably affected their occupations. These include mainly tourist service providers, shopkeepers, small vendors etc. Four percent did not respond while remaining feels unaffected by the establishment of Park.
- About 99 % of the people interviewed were aware about the purpose of tourists visiting Wandoor. Of them, 65 % knew that tourists were coming for viewing marine life, 33 % think that they come for enjoying beaches and nature and 1 % think that wildlife is the major attraction for tourists.
- 95 % view the Park authorities' behaviour towards them to be good and supporting. They expressed that there is regular interaction between them and Park authorities. Five percent (mainly fishermen) complained about occasional harassment.

Following were the main reasons for discont among the local communities:

- Ban on fishing inside the MNP
- No proper berthing place for fishing boats
- Occasional harassment by Forest/Police personnel within MNP
- Assurance of development made at the time of creation not fulfilled
- After Tsunami
    - Fish catch declined tremendously
    - Agricultural land also affected
    - No alternative means of livelihood except Tourism related jobs
- Recent restriction imposed on tourist boats plying to Mahua Dera beach, which is a new site with a beautiful beach and rich coral diversity attracting a large number of tourists. The site has been closed temporarily, which generated a lot of discontent since a number of fishermen turned tourist service providers had taken loans to buy motorized boats for tourism and faced difficulties in repaying loans due to the ban.

On interviewing people about their expectations from the Park authorities, they expressed following points:

- Permission within MNP
  - To carry tourists in small boats/country boats
  - For fishing
- Safe fishing routes through MNP to be demarcated
- Opening of Mahua Dera Beach for tourism through local tour operators
- Permission for country boats to carry eatables for sale to tourists within MNP
- Employment of local people as
  - Tourist Guides/Guards
  - Labourers
- Permission to open Shops and Restaurants near entrance of the Park

It was revealed during the interviews with Park authorities that they have, after a series of meetings with local village leaders, initiated following actions/measures:

- Safe passage routes for fishing boats through MNP has been demarcated
- Alternative berthing site for fishing boat has been identified outside MNP in consultation with stakeholders
- Fish landing facility is being developed by Fishery Department
- Additional Island (Alexendra) has been opened for tourism
- Formation of eco-development committee is in final stage
- Employment of local youth in seasonal works
- Agreed to
  - provide space to small vendors to run their shops near the entrance of Park
  - provide training and employing unemployed youth as tourist guides inside MNP
  - open Mahua Dera for tourism
  - permit boats of local tour operators inside MNP after clearance of certain legal requirements
- These initiatives will be within the legal framework of existing Acts and Rules

It is evident from the survey done that the potential of tourism is realized by local people. As a result they are identifying new areas for tourism and also making investments. However, they need support of the Administration, particularly the Forest Department. In response the Authorities have initiated people's involvement in conservation efforts through alternative livelihood options.

Acknowledgement

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Integrated economic valuation in coral reef management: Demonstration, appropriation and utilization of coral reef economic values for sustainability and conservation

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Abstract Underappreciation of the true economic value of coral reef resources is a major problem in developing countries like the Philippines leading to a lack of accounting and accountability in policy and development decision-making. This paper aims to highlight the vital need to integrate economic valuation in coral reef management and in integrated coastal zone management. It uses the total economic value framework, and argues that much of the economic values of coral reef resources have not been properly demonstrated and expressed in the market and policy making, thereby resulting to over exploitation, damage and decimation of coral reefs. Mechanisms on how to demonstrate such economic values (like entrance fees, conservation fees and others) and policies towards their capture are crucial aspects of integrated economic valuation. A few cases in the Philippines show that this is possible and can provide a good framework in helping attain sustainability and conservation of such national treasures.

Key Words: integrated economic valuation, coral reef management, economic values

Introduction

Coral reefs are highly productive and valuable marine resources and home to thousands of species. They provide habitats and food sources for countless organisms and reef-based tourism/ eco-tourism is a major source of livelihood for various coastal communities. Other benefits include coastal protection, biodiversity and the reefs' value as climate change indicators. The economic value of coral reefs is important, from direct use to indirect use and non use values (Table 1; Cesar 2000; Subade 2005).

However, coral reefs are threatened by over fishing, destructive fishing, coastal development, marine-based pollution, climate change impacts, and many other global and local factors. Philippine coral reefs cover an estimated area of 27,000 sq km, and are considered to under threat (BFAR-NFRDI-PAWB 2005). A large proportion of the impact stems from anthropogenic causes, caused by the very high population density of the country. Over-fishing, destructive fishing practices (blasting and the use of toxic substances) and sedimentation top the list of these impacts.

Thus, most of the country's coral reefs are in a degraded state, with over 70% with poor or fair quality and quantity of coral cover; and only 5% in excellent condition (BFAR-NFRDI-PAWB 2005).

Most reef areas in the Visayan Sea Basin, and in gulfs and bays are heavily overfished. In the Visayan seas, particularly in protected areas, poaching and fishing-related threats are the worst threats to coral reefs, followed by sedimentation, tourism related-activities, crown-of-thorns starfish infestations, and coastal development. Fishing and gleaning on coral reef areas account for 10-15% of the country's total fish catch (BFAR-NFRDI-PAWB.2005).

Table 1. Economic Values of Coral Reefs

DIRECT USE VALUES	INDIRECT USE VALUES	NONUSE VALUES

Direct benefits Goods & Services, Usually marketed values, like fish, sea cucumber, and scuba diving (with dive fees)	Functional benefits Environmental and ecosystem values - nutrient retention - flood control - storm/ coastal protection -external ecosystem support	Bequest Existence Option Quasi-option

A recent compilation of studies through time by the Philippine Coral Reefs Information Network (Philreefs 2008), showed mixed trends on the country's coral reefs indicator such as hard coral cover, fish biomass and fish abundance. Generally marine protected areas (MPA) sites have had increasing or no net change trend, while most non-MPA sites have had decreasing or no net change trend.

Usual Valuation Problem

Failure to account adequately for their economic values in development decision making could be considered a major reason for coral reef decline. Since natural and managed environmental/ marine resources are not bought and sold on markets, they are generally ignored in private and public development decisions. Coastal development is often preferred over pristine/preserved coastal habitats because only market values are considered. Inclusion of non-market values might have caused many coastal development projects appear less attractive investment options.

Reef fisheries are an over-capitalized sector where resources are invested to give lower net benefits. Because environmental/degradation costs are usually not included in cost/benefit calculations, the returns even from an over-fished fishery appear higher than they are in reality. Another undervaluation example is destructive fishing - fines and penalties are not based on economic values of damages caused, thus the activity persists.

Need to Integrate Economic Valuation in Coral Reefs Management

Coral reefs management therefore should not be devoid of economic valuation. Moreover, coral reefs research needs to include economic valuation as an integrated component and not just an after thought.

Economic valuation involves three major phases (Fig. 1). First demonstration of coral reef economic values (Georgiou et al. 1997). Then appropriation, which is capture of coral reefs economic values through appropriate policies and mechanisms (Georgiou et al. 1997). Third is utilization, which concerns the use of measured coral reef economic values and/or captured coral reefs economic values in IEC, decision making and in financing of conservation

The economic valuation of coral reefs, i.e. the demonstration, appropriation and utilization of their economic values is a crucial impetus to attaining their sustainability and conservation.

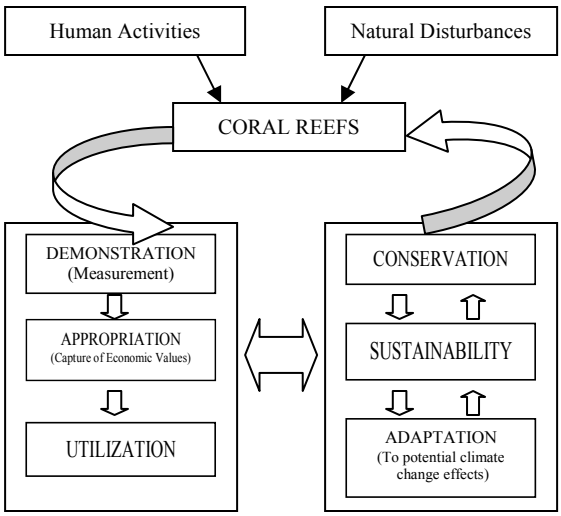

Figure 1. Integrated economic valuation in coral reefs management

Integrated Economic Valuation of Coral Reefs

Improving coral reef management, in the context of Integrated Coastal Resource/Zone Management involves the integration of economic valuation and makes integrated economic valuation or (IEV) an inseparable part of sustainable ICZM or ICRM. IEV provides researched-based incentive/ disincentive mechanisms to: (i) attain sustainability, and (ii) generate financing source for conservation and / or coastal resource management. IEV does not end at mere study/ measurement of economic values but provides mechanisms to capture or appropriate them. IEV transcends policy analysis towards policy advocacy.

In the ICZM or ICM developed at various sites by the UNDP-funded Partnership in Environmental Management of the Seas of East Asia (PEMSEA), economic valuations were embedded in various stages such as: preparing, initiating, developing, adopting, implementing, refining and consolidation of management plans (www.pemsea.org).

Examples of IEV in Coral Reef Management

The integration of economic valuation in coral reef management is evident in the examples of Mabini and Tingloy, Batangas and the Tubbataha Reefs

National Marine Park, a UNESCO World Heritage Site, where scuba diving tourism is a major activity.

Economic valuation was undertaken by a WWF-Philippines-led team to demonstrate and appropriate coral reef economic values mainly through divers' fees. A conservation fee surcharge or an annual diver's pass was collected from divers (Padilla et al. 2005). The IEV process framework involved: Project design, marketing the project to LGU officials & stake holders, research /surveys, sectoral briefings & consultations, drafting of ordinance, stakeholders consultations, refinement & finalization of draft ordinance, provincial approval of the ordinance, IRR formulation, implementation, monitoring & evaluation. Over a period of eight months (September 2003 to May 2004) a total of 1.3 million pesos was collected from conservation fees. Proceeds from the collection were used for incentive rebates to boatmen (who are fishers) and for financing law enforcement through the Bantay Dagat (Sea Watch) that protected coral reefs and other coastal resources.

At Tubbataha Reefs NMP, a similar process of economic valuation was also undertaken, though the implementing rule was for the Tubbataha Management Office (TMO) to collect the conservation fees both from the divers and the boat operators. A "willingness to pay" survey was undertaken that determined the entrance fees and conservation fees per diver as follows US$50 for foreign divers and US$ 25 for local divers : (Mejia et al. 2000; Tongson and Dygico 2007)

The realized revenue is allocated among a seed fund for conservation, core park administrative costs, and livelihood of adjacent communities in Cagayancillo, Palawan.

Resource Rent for Resource Conservation

Resource utilization generates resource rent (benefits or revenues less costs), alternatively called economic profit or profit. This rent rightfully belongs to the resource, and should be used to conserve it and allow it to replenish, thereby attaining sustainability.

ICZM should therefore incorporate IEV which is a major factor (perhaps "the key?") towards successful and sustainable ICZM (MPA). IEV can assure MPA sustainability and thereby provides sustainable financing mechanisms for marine conservation.

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Towards Local Fishers Participation in Coral Reef Monitoring:A Case in Tingloy, Batangas, Philippines

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Abstract. The five-island municipality of Tingloy is a popular diving site known for its rich coral reefs. However, these reefs are being threatened by man-made stresses and natural disturbances. A monitoring framework was developed through the integration of scientific and indigenous knowledge in determining and assessing the present status of coral reef environment. One of the benefits of the developed monitoring framework is the empowerment of the fishermen. Institutions and fishermen in the area in cooperation with the academe provided the major backbone of the framework. The fishermen were interviewed, trained and participated in the whole year monitoring of the coral reef environment. The monitoring framework developed served as an overall guide for monitoring activities and the data gathered served as the baseline information of the coral reef environment in the area.

Key words: coral reefs, fishermen, monitoring framework, baseline information, Tingloy

Introduction

The Philippines is blessed with one of the most diverse coral reef ecosystems in the world as it lies in the region known as "The Coral Triangle". However, Philippine coral reefs are increasingly threatened ecosystems. Coral cover is rapidly declining, fish populations are low and reef productivity dropped by one-third during the last decades (Jameson et al. 1995). The damage that occurs to coral reefs is usually the result of natural and man-made interference.

In Luzon, one of the most extensive coral reef ecosystems which is a favorite destination of scuba divers is found along the municipality of Tingloy, Mabini and its vicinity. Tingloy is located in Maricaban Island, Batangas, 120 km south of Manila. Tingloy is situated in the southern coast, and the coral reefs are found along Batangas Bay and Maricaban Strait . The western side of Tingloy (e.g. Sepoc point., Sombrero and Caban islands), is considered one of the best diving sites in the area (Uychiaoco and Aliño 1995). The coral communities of Mabini and Tingloy are said to have traditionally supported rich near-shore fishing and in recent years, a growing tourism industry. In the early 80's however, increased fishing effort using destructive methods, uncontrolled development of the land, increased visitation by scuba divers and day-trippers and increased pollution (solid and liquid waste), began to threaten the coral reef ecosystems of these areas *(*White and Vogt 2000).

The WWF-Philippines (World Wide Fund for Nature) has been implementing conservation activities in Mabini and Tingloy since 1998. It started by setting up community-based, multi-sector inter-municipal council (MATINGCADC), which it envisions will eventually manage the area's rich marine resources. To further ensure this, the project strengthened the coastal law enforcers, "Bantay Dagat" that the local council has organized. With the success in the enforcement efforts of Tingloy to curb illegal and destructive fishing activities, members of the municipal council, Sangguniang Bayan who are also members of the local council, MATINGCADC, are now considering on identifying and declaring certain areas within their political jurisdiction as community-managed municipal marine reserves (Dumaop 2000).

For the past 20 years, much has been done on coastal and reef management in the country, involving government, non-government organizations, local communities and resource users with emphasis on communities' participation in the rehabilitation and conservation programs (White and Vogt 2000; Gutierrez et al. 1996; Fernandez, Matsuda and Subade 2000). There has been an increasing level of participation by local communities and resource users, particularly the fishermen in coastal management across the

country, as evidenced by several cases such as the stories of Apo Island, Banate Bay (Fernandez et al. 2000) and others. In most of those projects, coastal management and monitoring has been done mainly by scientists, and focused on biophysical and chemical aspects. Moreover, most of the monitoring guidelines and techniques were determined by the scientists. This situation makes it difficult for fishermen to be actually involved in monitoring activities. Thus, local participation would usually be limited to patrolling while monitoring would be largely done by the scientists.

There is therefore a need of encouraging participation down to the grassroots level for monitoring and assessment since it is through this that one could determine the status of the coral reef ecosystem and the effectiveness of a policy, a program, or management activity being implemented. This study aimed to facilitate and encourage community participation in the development process of the integrated methodological framework for environmental monitoring of coral reefs in Tingloy, Batangas. It also integrated scientific and indigenous knowledge in determining and assessing the present status of coral reef, reef fish and water quality in the area. The framework developed could also serve as an overall guide for the monitoring activities that was undertaken by the coastal community. The development of methodological framework for environmental monitoring of coral reefs in Tingloy, Batangas, provided a good venue for the expression of community's knowledge and participation in the formulation of monitoring plan for the management of their marine resources.

The Research Process

This study was conducted in the five islands of Tingloy, Batangas involving the community and different institutions for the rehabilitation and conservation of marine resources. Figure 1 shows the conceptual framework of the study. The first component is the community which composed of the fishermen, who are the main residents, and the local government unit (LGU) in the area. The second component is the organization of institutions that were equally important such as the Provincial Government Environment and Natural Resources Office (PGENRO), the academic institution and the Kabang Kalikasan ng Pilipinas or the WWF-Philippines. The PGENRO and the academe provided the scientific knowledge in the environmental assessment while the role of KKP-WWF was measured through its past and present efforts as well as future plans for the sustainable management of the aquatic ecosystem. The third component is the coastal environment specifically the extensive coral reef ecosystem of Tingloy.

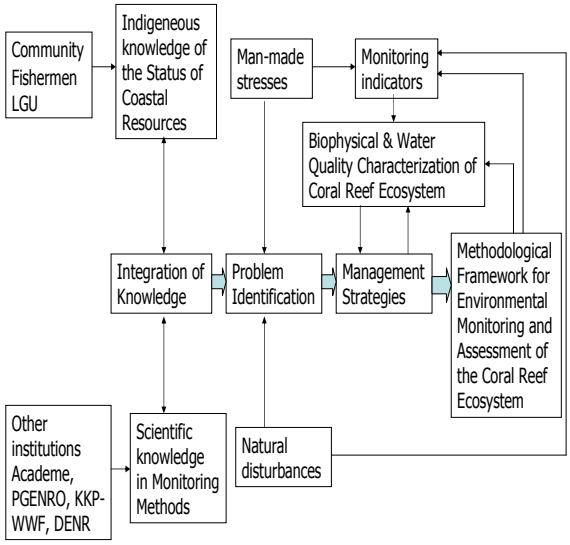

Figure 1. Conceptual framework of the Study.

The framework could be viewed as a system with several components, interacting to come up with an integrated methodological framework for environmental monitoring. The methodological processes for the development of the said framework were shown in Figure 2.

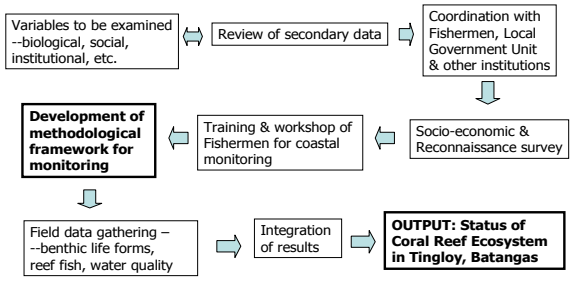

Figure 2. The research process for the development of the framework.

The individual survey was conducted over a 5-week period from June to July 2001 in Tingloy, Batangas. To gather the needed data, a structured and open-ended interview schedule was administered to the fishermen after it was translated to Tagalog and pre-tested in Barangay Sulo, Mabini. The questionnaire consisted of 10 pages divided into the following five sections: 1) demographic and socio-economic characteristics of

fishermen, 2) fishing and resource management practices including fishing effort and catch, 3) knowledge, 4) perception, and 5) attitude. In the last part of the questionnaire, the fishermen were asked if he is willing to participate in monitoring of coastal resources and the reason for his answer.

A two-day training on coastal monitoring was conducted for the fishermen. Out of the 109 fishermen interviewed, eight participated in the training-workshop. The five criteria for the selection of participants in fishermen's training were: 1) his willingness to volunteer without payment in the monitoring, 2) have at least a total score of 100 out of 128 points (on the perception, attitude and knowledge questions), 3) must agree or strongly agree to the establishment of marine reserves, 4) physically fit, and 5) able to read and write. One of the limitations in the number of participants is the limited funds considering that the activity only provided food and transportation expenses and no extra allowance was given.

The data from the social survey provided input in the identification of the major problems to be addressed in monitoring. Out of the 26 problems identified, six major problems were identified with the fishermen for environmental monitoring The six major problems identified were poverty or low income among the municipal fisherfolks, overfishing or overexploitation of resources, habitat degradation due to dynamite and cyanide fishing, pollution due to the presence of considerable amount of garbage and oil in the coastal waters, and storms or typhoon.

Based on the results of the reconnaisance conducted, five (5) sampling sites were identified for coral cover, reef fish and water quality assessment of the study. The five sites selected can give a representative estimate of the status of the coral reef ecosystem in Tingloy, Batangas. The criteria for the selection of the sites are as follows: 1) serve as a site of baseline data of the status of coral reefs in Tingloy 2) will serve as one of the major sites for future monitoring activities 3) serve as one of the possible sites for the establishment of marine reserve and 4) serve as one of the possible sites for protection and rehabilitation programs. Fishermen preferences of the sites as a fishing ground and where oil spill was observed were also considered. The results of manta tow survey was integrated in the focus group discussion to finalize the selection of sampling sites and appropriate sampling time for the wet and dry season of various physical, biological and chemical parameters of the study.

Coral and reef fish surveys were done during the month of March. Two fishermen were assigned for each site for the coral and reef fish survey. A line-intercept technique and snorkel survey were used by the divers and fishermen, respectively in the assessment of coral reef and reef fish. Based on Gomez and Alcala (1978) studies, coral cover was categorized as Excellent (75-100%), Good (50-74.9%), Fair (25-49.9%), and Poor (0-24.9%).

Water sampling of coastal waters was quarterly scheduled in the months of August, November, February and June. Sample collection for each month was carried out by two fishermen for all the five sites over a one day period. Microbial, physical and chemical attributes of the coastal water were determined with assistance from PGENRO.

Methodological Framework for Monitoring

The framework was developed through the participation of the community particularly the fishermen and various institutions (Table 1).

Status of Coral Reef Ecosystem

The methodological framework was then applied to determine the status of the coral reef ecosystem in Tingloy, Batangas. Five sites were monitored for coral cover, reef fish and water quality.

Coral Cover

Sites chosen for the survey were the Caban reef, Macawayan Reef, Bonito Reef, Pisa Reef and Sto Tomas Reef. A coral reef assessment using the LIT by divers and snorkel survey by fishermen. Gomez and Alcala (1978) categorization was used.

All five sites were categorized in fair condition by the fishermen survey. On the other hand, diver's survey showed only two sites, Caban reef and Makawayan reef in good condition while the rest of the sites were in fair condition. Both sites had high live hard coral cover among the selected sites thus it can be said that areas in Caban and Makawayan are recommended sites for establishment of marine reserves.

Fish Biomass

Among the five sites, Caban reef (59428.21g) has the highest fish biomass while Pisa reef (12,372.34g) has the lowest. Other reef areas are as follows; Bonito reef (49900.34g), Makawayan reef (36222.93g) and Sto. Tomas reef (25183.46g). Fish biomass could not be determined from the fishermen survey because it requires that fish be identified at the species level.

Table 1. Integrated methodological framework for environmental monitoring of coral reefs ecosystem and social system in Tingloy, Batangas.

ISSUE OR PROBLEM	CAUSES	POSSIBLE INDICATORS	MANAGEMENT STRATEGIES	WHERE TO MONITOR	WHEN TO MONITOR	MONITORING METHOD	PERSON(S)/ INSTITUTION INVOLVED
Poverty or low income of municipal fisherfolk*	Too many fishermen, overexploited resources, low catch, management practices	Income*, no. of fishers*, health condition	Alternative livelihood*, fishing regulation through licensing	Per municipal barangay	Once a year	Social survey	LGU, Academe, NGO, DENR
Overfishing*	Changes in fishing effort, natural disturbances, changes in coral cover, management practices	Fish sizes and abundance	Harvest regulations (MPA/Zoning, seasonal closure, gear restriction, species restriction), patrolling and enforrcement*	Inside and outside MPA, representative sites of the Tingloy	Wet and dry season	Fish visual census	Fishermen, Academe, NGO
	Increase in fishing effort, management practices like MPA enforcement	Fishing effort; catch per unit effort*		Per municipal barangay	At least once a month	Fish catch monitoring	Fishermen, Academe, LGU, NGO
Habitat Degradation*	Destructive fishing methods, natural disturbances, management practices like MPA	Coral cover*	Education campaign, patrolling & enforcement*, harvest regulations (MPA, seasonal closure, gear restriction, species restriction)	Inside and outside MPA, representative sites of Tingloy	Once a year	Manta tow, snorkel survey	Fishermen, Academe, NGO
Pollution (solid waste and sewage)*	Waste disposal practices, management practices	Garbage*	Waste management (proper disposal system), Patrolling and enforcement*, Information campaign for proper sanitation	Representative sites of Tingloy	Every quarter of the year	Water quality analysis particularly on ammonia, nitrogen, phosphate, DO and BOD	Fishermen, PGENRO, Academe, NGO
	Lack of toilet facilities, management practices	Cases of diarrhea		Representative sites of Tingloy especially Sto. Tomas site.	Every quarter of the year	Water quality analysis particularly on Total and Fecal Coliforms	Fishermen, PGENRO, Academe, NGO
Pollution (oil spill)*	Industry and ships oil spill, management practices	Oil spill*	Lobbying for waste reduction from oil refineries and ships*, patrolling and enforcement	Representative sites of Tingloy especially Sto. Tomas	Every quarter of the year	Water quality analysis particularly on Oil and Grease	Fishermen, PGENRO, Academe, NGO
Pollution (sedimentation)	Loss of mangrove areas, deforestation, management practices	Coral cover	Mangrove reforestation, replanting of trees or vegetation in the upland	Representative sites of Tingloy especially Sto. Tomas	Once a year	Manta tow, snorkel survey	Fishermen, Academe, NGO
	Same as above	Siltation		Same as above	Every quarter of the year	Water quality analysis especially TSS	Fishermen, PGENRO, Academe, NGO
Storms and/or global warming*	Natural disturbances, increasing CO_2 level	Coral cover*	Reduce man-made stress to enable the environment to recover more easily	Representative sites of Tingloy particularly Bonito	Once a year	Manta tow, snorkel survey	Fishermen, Academe, NGO

Legend: * - identified by fishermen

Fish Abundance

About 31 families were recorded by the divers in all the five sites while the fishermen identified 25 families. Survey showed that Makawayan reef has the highest fish abundance, followed by Caban reef. Pisa reef and Sto. Tomas reef has the lowest fish abundance. By family, Pomacentridae had the largest contribution to the mean biomass, accounting for more than 30% of the total biomass for each site. It is also the most numerous fish group per individual counts for all sites. Fishermen's survey on fish abundance per individual counts was also highest for Pomacentridae.

Fishing Effort and Species Caught

Most common fishing gear used was hook and line, aboard small and mostly non-motorized boats. Fishing was done almost everyday averaging 3-5 hours. Fish caught were mostly small pelagics such as skipjack tuna, mackerels and scads.

Coastal Waters of Tingloy, Batangas

In general, the water quality of coastal waters of Tingloy was still within the DENR standard for Class SA coastal and marine waters except for some areas in the total and fecal coliforms standard. Bonito and Sto Tomas are way beyond the maximum DENR standard for coliforms. All sites have high total suspended solids value for all quarters which indicate the degree of sedimentation in Tingloy. Among the five sites, the coastal waters in Sto. Tomas exceeds the allowable limit for Class SA marine water. Sto. Tomas has the highest readings in oil and grease, BOD, and fecal coliforms. It also has a high reading in total suspended solids and total coliforms. The level of oil and grease in Sto. Tomas showed that Tingloy is not spared from oil spill in Batangas. With these results, resort establishment is not advisable along the coast of Sto. Tomas.

Conclusion and Recommendations

The development of methodological framework for environmental monitoring involved the participation of coastal communities (i.e. fishermen) and institutions. However it appears that the fishermen need more practice for biological survey of benthic lifeforms as well familiarization with the scientific description of these substrate cover. In addition, consultation with the fishermen are necessary to improve the existing method or in trying another method like the point-intercept transect, whichever suits their capability.

In retrospect, the benefits in the development of the methodological framework are the following: (1) The community and institutions are given a chance to participate and share their indigenous and scientific knowledge; (2)The fishermen are empowered in the development process of the framework; (3)The baseline characterization of the coral reef ecosystem is determined through the combined efforts of the community; (4) The framework could serve as a guide that can be applied by other coastal communities. Modifications may be made due to differences in human activities in the area.

Limitations are as follows: (1) Its application in other areas may be limited by the capacity of the fishers in the area. Thus training of these fishermen should be considered; (2) Its application would require going into the different components of the framework and this would entail time and money. However, this framework could give a starting point for the development of other methodological framework in other communities.

(3) Not all indicators were monitored due to time and financial constraint; (4). In this study, some local names of reef fishes were not identified. It is therefore recommended that a study be conducted for a uniform identification of reef fishes with its local name, English name, and scientific name.

Acknowledgement

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Are the Coral Reef Finfish Fisheries of South Florida Sustainable?

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Abstract. We used population abundance and size structure data from fishery-independent (visual census) and fishery-dependent (creel surveys) monitoring programs from the Florida Keys and Biscayne National Park to estimate stock mortality rates and current reproductive potentials of the seven most commonly harvested reef fishes. Numerous indicators revealed these reef fishes are currently experiencing unsustainable rates of exploitation. Annual growth in recreational fishing effort compounds this problem. If healthy reefs are Florida's future, exploitation of reef fish stocks must be reduced. Fishery management actions were evaluated that could possibly reduce fishing mortality and increase reproduction potential sufficiently to achieve sustainable stock conditions. Results indicated that, when using only traditional management approaches such as increased minimum harvest sizes or decreased bag limits, rather radical changes will be needed to achieve sustainable stock conditions, and any improvements may be negated by continual increases in fishing effort over relatively short time horizons. We conclude that, in addition to traditional fishery management controls, contemporary measures such as placing a portion of the population under spatial protection will likely be needed to achieve long-term sustainability of Florida's coral reef finfish fisheries.

Keywords: Florida, coral reefs, overfishing, sustainable fisheries

Introduction

The Florida Keys coral reef ecosystem, including Biscayne National Park, is inhabited by more than 400 fish species and supports multibillion-dollar fishing and tourism industries. Over recent decades, reef fish populations have declined owing to a variety of human-related stressors, most notably fishing and habitat alterations (Bohnsack and Ault 1996; Ault et al. 1998, 2005a). These fishes are intensively exploited (Ault et al. 1998, 2001, 2005b) by a rapidly growing human population and recreational fishing fleet (Fig. 1). Biscayne National Park (BNP) is in the process of developing plans to guide resource management decisions for the Park over the next 15-20 years that will contribute to conservation of fish species and their habitats, and sustain a tradition of quality fishing experiences for generations to come. The strategy for fisheries management is being developed cooperatively by BNP and the Florida Fish and Wildlife Conservation Commission (FWC), with input from members of government agencies, area universities, and the public. Fishery management concerns for reef fishes in south Florida are two-fold: (1) declines in the abundance of fish; and, (2) loss of quality fishing opportunities.

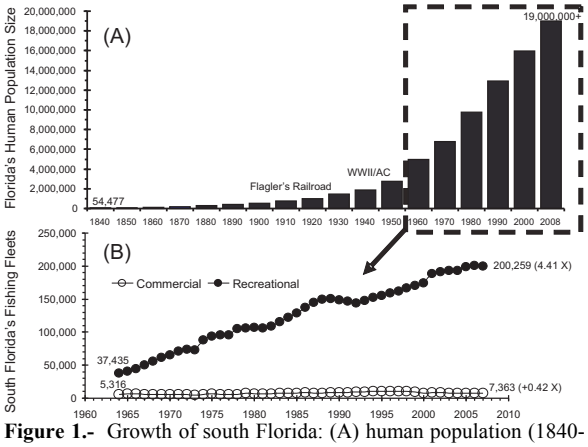

Figure 1.- Growth of south Florida: (A) human population (1840-2008); and, (B) fishing fleets (1964-2007).

To mitigate reef fishery declines, the South Atlantic Fishery Management Council in 1983 and the Florida Marine Fishery Commission in 1984 began establishing minimum size, season, and bag limit restrictions on a number of reef fish species. The current size and bag limit regulations for reef fishes in south Florida have been in place since the late 1990s (www.myfwc.com/marine/regulation.htm). However, the most recent assessments suggest that majority of snapper-grouper species are currently

fished at unsustainable levels (Ault et al. 2005b).

To assist in the BNP management processes, in this paper we assessed the sustainability of key reef fish stocks in BNP in relation to the Florida Keys, Once exploitation levels were identified, we evaluated potential benefits of more restrictive size and/or bag limits in terms of their efficacy to achieve sustainable populations and meet regional resource management goals.

Methods

Data Sources.- Two principal data sources were used, fishery-independent and fishery-dependent. Fishery- independent data were visual census surveys of reef fish species abundance and size structure for the Florida Keys ecosystem, including Biscayne National Park, for the period 2000-2004 (Bohnsack et al. 1999; Ault et al. 2001, 2005b). Fishery-dependent data were creel census surveys conducted in BNP for exploited species abundance and size structure for the two time periods, 1995-1998 and 2000-2004 (Ault et al. 2007).

Sustainability Analysis.- The principal stock assessment indicator variable we used to quantify sustainability status was average length ($\overline{L}$) of the exploited part of the population, the interval between the minimum size/age of first capture (L_c/a_c) and the maximum size/age (L_λ/a_λ) observed in the stock. For exploited species, $\overline{L}$ directly reflects the rate of total instantaneous mortality through alterations of the population size structure (Beverton and Holt 1957; Ehrhardt and Ault 1992; Quinn and Deriso 1999).

Estimates of average length were obtained from length composition data derived from the fishery-independent and -dependent data following the procedure described in Ault et al. (2005b, 2008). Using estimates of $\overline{L}$, total instantaneous mortality rate Z was estimated using the method of Ehrhardt and Ault (1992) and an iterative numerical algorithm (computer program LBAR, FAO 2003). Life history parameters for maximum age, growth and maturity for the reef fish species considered are given in Ault *et al.* (1998, 2005*b*). Additionally, we evaluated the two major components of Z, namely fishing mortality rate F and natural mortality rate M. In this process, we estimated M from lifespan, and F was estimated by subtracting M from Z (Ault et al. 1998).

A numerical cohort-structured model (Ault et al. 1998, 2008) was used to compute several fishery management reference points of stock status, or "*sustainability benchmarks*", including yield-per-recruit (YPR), spawning potential ratio (SPR), and limit control rules. Spawning potential ratio (SPR) is a management benchmark that measures a stock's potential to produce yields on a sustainable basis, and is computed as the ratio of current SSB relative to that of an unexploited stock. Benchmarks used to evaluate sustainable exploitation as a limit control rule were: F_{msy} (F generating maximum sustainable yield, MSY); B_{msy} (population biomass at MSY); and SPR (spawning potential ratio). We defined F = M as a proxy for F_{msy} (Quinn and Deriso 1999).

Bag & Size Limit Analysis.- We used the BNP creel census data to facilitate evaluation of bag limits. Catch and effort data were computed in terms of landings per angler-trip (i.e., number of fish landed and kept per person-trip). Current bag limits were evaluated in terms of percentages of trips that caught more than specific amounts of fish per person.

To evaluate potential gains in population benchmarks through increases in the minimum size of first capture, we conducted a "eumetric" fishing analysis (*inter alia* Beverton and Holt, 1957) for each key reef fish species. This analysis identifies the optimum combination of minimum size of first capture L_c given a particular fishing mortality rate F that results in maximal yields in weight and/or numbers of fish.

Results

Estimates of $\overline{L}$ from BNP creel data for seven principal exploited reef fishes were consistent between the 1995-1998 and 2000-2004 time periods (**Table 1**). The estimates of $\overline{L}$ from the latter time period were consistent between the BNP creel and Florida Keys visual census (Fig. 2). A substantial proportion of fishes observed in the creel census were smaller than the minimum legal size (Table 1). These undersized fish were not included in the computation of $\overline{L}$.

These data, together with the known life history parameters for each of these species, were then used to calculate estimates of current fishing mortality rates, stock biomass, SPR and YPR for each of the key reef fish species. Values of the F/F_{msy} ratio plotted against the B/B_{msy} ratio (Fig. 3) suggest all seven species are being subjected to unsustainable rates of exploitation (F-ratio >1, B-ratio <1; Ault et al. 2008).

We evaluated creel census catches to determine the closeness of catch rates to current bag limits (Table 2). The majority of the hook-and-line trips caught none of the key reef fishes, and only a small fraction of these trips ever reached the current bag limit. The only exception was for hogfish taken by spearfishers, who reached the bag limit on about 10% of the trips.

Given that we found that all seven of the key species are currently being subjected to unsustainable rates of exploitation, we evaluated the potential

impact (in terms of SPR and YPR) of changing the size limits. For these analyses, we assumed that current estimated fishing mortality rates would continue unabated at their most recent levels, and then we increased the minimum size limit to the apparent optimal (i.e., eumetric) level.

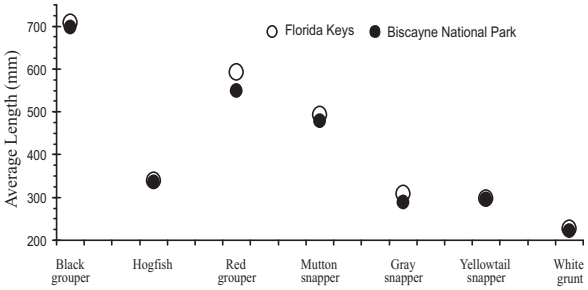

Figure 2.- Average size in the exploited phase for 7 reef fish species in the Florida Keys and BNP.

Summary results of these analyses for the "eumetric" minimum harvest size are given in **Table** 3. The changes in size correspond to a 2-3 fold increase in the age of first capture for most species. This increase in size/age is projected to favorably increase SPR for all stocks to above the 30% SPR federal standard for sustainability.

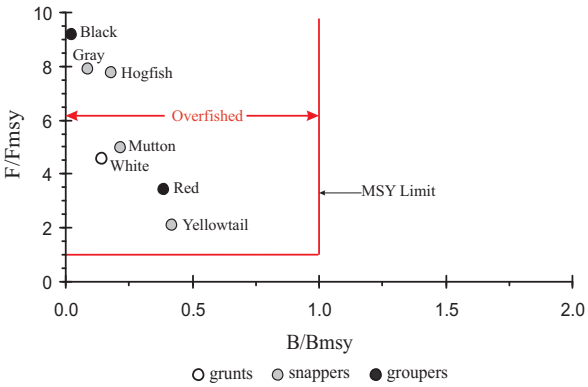

Figure 3.- Plot of F/Fmsy ratio against B/Bmsy ratio for 7 key reef fish species in the Florida Keys-BNP coral reef ecosystem for the years 2000-2004.

The time required for reaching the new equilibrium following implementation of a size change varies among the species, but could be a decade or longer for groupers (Fig.5).

Discussion

There has been a continuous long-term increase in registered recreational boats and fishing effort coupled with human population growth in South Florida. In the last 15 years, the recreational fleet has increased from about 140 thousand registered vessels to over 200 thousand (Fig. 1) and there is nothing to suggest that recreational fishing effort will not continue to increase into the future within the park and the greater south Florida area. Unless some additional restrictions are placed on the recreational fishery, fishing mortality rates for all of the species are likely to continue to increase into the future.

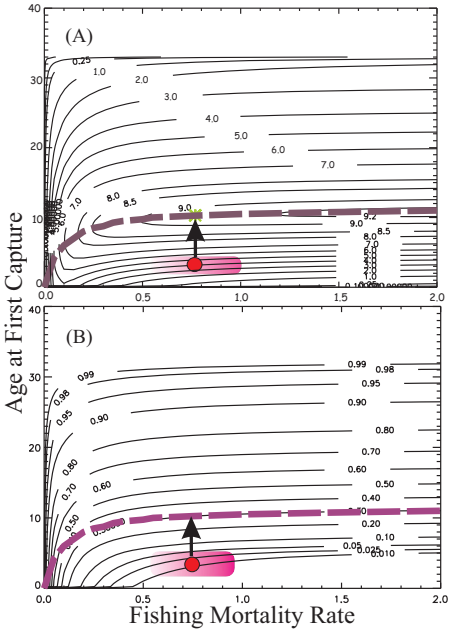

Figure 4.- Black grouper isopleths for: (A) yield-per-recruit in weight (Yw/R); and (B) spawning biomass-per-recruit (SSB/R). Current estimated *F* is shown in the shaded area of each panel, and the arrows denote required changes in size to reach the eumetric line (dashed).

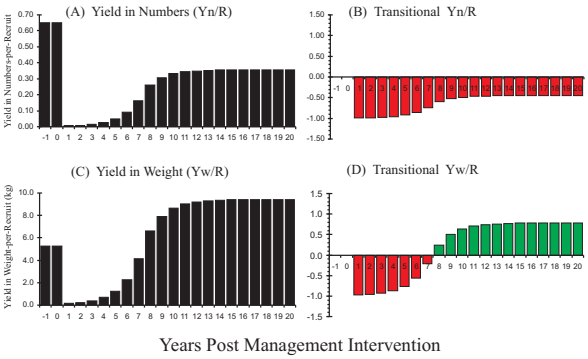

Figure 5.- Transitional dynamics and difference from initial equilibrium for Florida Keys black grouper: (A-B) yield in numbers-per-recruit; and, (C-D) yield in weight-per-recruit, after a eumetric size-limit change.

All seven species analyzed in this study were found to have fishing mortality rates that exceed those which are generally considered sustainable for healthy fish populations. While there has been an apparent stabilization of effective fishing mortality over the past decade, perhaps in response to the various management controls, none of the BNP (or Florida Keys) reef fish populations equaled or exceeded the

minimum 30% spawning potential ratio (SPR) standard generally accepted as necessary for a sustainable fishery stock.

Even though the average number of fish landed per fishing trip for all of the species analyzed was found to be very low, we found a reduction in bag limit may, in some cases, reduce fishing mortality rates. In cases where catch rates are principally low (e.g., black and red grouper, and mutton snapper), reductions in bag limits would have little apparent effect, or would close the fishery. Our analysis suggests that a reduction in bag limits alone will not be sufficient to allow stocks to recover.

On the other hand, increasing the minimum size of first capture could lead to demonstrable increases in yield and in stock spawning potential. The important point suggested by our analyses is that a dramatic increase in the size at first capture is required to move stock spawning levels above those considered minimum for sustainability. This would, *de facto*, make the entire fishery a closed area for several years following the change (**Fig. 5**).This is particularly true for black grouper, hogfish, mutton snapper, gray snapper, and white grunt.

With either increases in minimum size or decreases in bag limits there will undoubtedly be an unintended increase in catch-and-release mortality incurred as both undersized fish and fish in excess of the allowable bag are released. This topic has been thoroughly reviewed by Bartholomew and Bohnsack (2005), who reported that catch-and-release mortalities ranged between 10 and 90% for over 200 species studied. For reef fishes targeted in BNP, release mortality rates would likely average from 10 to 30% from catch-and-release fishing.

Studies as early as Beverton and Holt (1957) and many others (Quinn and Deriso 1999) have shown that the ratio of natural mortality to growth rate of a species can provide a good indication of the rate at which species will recover when exploitation rates are reduced. All the reef fishes analyzed have relatively low M/K values. Unfortunately, this suggests that even if the necessary size were instituted immediately, stocks would not likely reach a sustainable status for at least 10 years.

In this paper we have only considered traditional management tools as the means of ensuring the sustainability of exploited reef fish stocks. While increased size limits have shown some promise, we believe that the only long-term solution will eventually involve multiple control methods including traditional approaches such as limiting the amount of fishing effort and/or restricting sizes to be captured, combined with more contemporary approaches like placing a portion of the population under spatial protection to ensure long-term resource sustainability.

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Table 1.- Biscayne National Park creel survey estimates of average size in the exploited phase, $\overline{L}$, and 95% confidence intervals (CI) for seven principal reef fishes for two time periods. Also reported is the percentage of measured fish (n) below the minimum legal size for the period 2000-2004; n/a denotes no legal size limit.

		2000-2004			1995-1998	
Species	L_c (mm)	n	$\overline{L}$ (95% CI) (mm)	Undersized (%)	n	$\overline{L}$ (95% CI) (mm)
Black grouper (*Mycteroperca bonaci*)	600	13	697 (648, 748)	23.5	31	691 (671, 710)
Red grouper (*Epinephelus morio*)	500	53	549 (537, 562)	29.3	64	535 (527, 543)
Mutton snapper (*Lutjanus analis*)	400	81	478 (462, 494)	36.7	48	499 (471, 527)
Gray snapper (*L. griseus*)	250	891	288 (286, 291)	13.7	979	292 (289, 294)
Yellowtail snapper (*Ocyurus chrysurus*)	250	644	296 (293, 299)	6.3	385	287 (284, 290)
Hogfish (*Lachnolaimus maximus*)	300	487	336 (333, 340)	21.7	492	347 (343, 352)
White grunt (*Haemulon plumieri*)	170	1126	221 (220, 223)	n/a	1878	217 (216, 218)

Table 2.- Bag limits (number of fish per person-day) for seven exploited species and the percentage of sampled trips in the Biscayne National Park creel survey that landed specified numbers of fish per person. Sampled trips are the number of boat trips using the specified gear that fished within habitats where a given species is found.

				Percentage (%) of Trips Landing:				
Species	Principal Gear	Sampled Trips	Bag Limit	>0 fish	≥1 fish	≥2 fish	≥5 fish	≥10 fish
Black grouper	Hook-line	1089	2	1.4	0.4	0.0	0.0	0.0
Red grouper	Hook-line	1089	5	5.2	0.9	0.4	0.1	0.0
Mutton snapper	Hook-line	1661	10	5.5	1.7	0.3	0.1	0.0
Gray snapper	Hook-line	1661	5	18.7	10.1	5.1	0.3	0.0
Yellowtail snapper	Hook-line	1089	10	15.3	8.6	5.2	1.7	0.4
Hogfish	Spear	169	5	81.7	60.4	36.7	9.5	0.0
White grunt	Hook-line	1089	none	18.9	14.1	9.3	3.2	0.6

Table 3.- Comparison of the estimated spawning potential ratio (SPR) at the current legal minimum size with the projected SPR at the minimum size corresponding to a eumetric fishing strategy for seven reef fish species. Also given are maximum age (a_λ), age at sexual maturity (a_m), and age at minimum capture size (a_c).

			Current			Eumetric		
Species	a_λ (y)	a_m (y)	min Size (mm FL)	a_c (y)	SPR (%)	min Size (mm FL)	a_c (y)	SPR (%)
Black grouper	33	5.2	610	3.4	0.8	1078	10.0	31.2
Red grouper	29	4.3	508	5.4	17.7	648	8.8	35.7
Mutton snapper	29	2.0	406	3.7	8.4	703	10.0	38.0
Gray snapper	28	2.0	254	2.3	3.1	545	9.5	35.4
Yellowtail snapper	14	1.3	254	2.4	14.1	292	4.3	46.5
Hogfish	23	0.8	305	3.3	6.7	569	10.5	44.2
White grunt	18	1.6	170	1.5	4.9	378	6.5	39.4

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Reproductive classification and spawning seasonality of *Epinephelus striatus* (Nassau grouper), *E. guttatus* (red hind) and *Mycteroperca venenosa* (yellowfin grouper) from The Bahamas

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Abstract. Fish of the family Serranidae: subfamily Epinephelinae are some of the most heavily harvested reef fish in the Caribbean. However, large knowledge gaps exist about their reproductive biology. Understanding a species' reproductive biology is critical for species management and provides evolutionary insight into species-specific reproductive strategies. Epinephelinae species possess a diversity of reproductive strategies, comprised of life-history traits which are highly variable between and amongst populations (e.g. size at sexual maturity, spawning duration, sex ratio). For this study, a reproductive histological classification system was refined for application to Bahamian fish populations of: *Epinephelus striatus* (Nassau grouper), *E. guttatus* (red hind) and *Mycteroperca venenosa* (yellowfin grouper). The use of a single classification system on multiple species provided a reliable framework to assess the status of reproductive life-history traits. Spawning seasonality in The Bahamas was described for populations of *E. striatus, E. guttatus* and *M. venenosa.* Spawning for *E. striatus* peaked November through January, while *E. guttatus* peaked in January and *M. venenosa* peak in March and April. The classification system will be used to establish consistent monitoring techniques and the results provide information for future management efforts in The Bahamas and allow for comparison to populations throughout the Caribbean.
Key words: Nassau grouper, red hind, yellowfin grouper, reproductive biology, gonad histology classification

Introduction

The reproductive biology of a fish is defined both by the combination of the species-specific reproductive mode and reproductive traits (Winemiller and Rose 1992, Murua and Saborido-Rey 2003, Morgan 2008). The reproductive mode does not vary between populations and is defined by the combination of the sexual development pattern (e.g. gonochoristic or hermaphroditic) and the gamete production system (e.g. determinate or indeterminate). Reproductive life-history traits (e.g. spawning seasonality and duration, age or size of sexual maturity, and sex ratio) vary between and amongst populations (Winemiller and Rose 1992, Murua and Saborido-Rey 2003, Morgan 2008). All are critical to understand a given population because they provide insight into how different strategies influence gamete production (Winemiller and Rose 1992) and how life-history trait plasticity can greatly alter a population's productivity or reproductive potential over time (Winemiller and Rose 1992, Morgan 2008).

Histological analysis of gonads provides more accurate and specific information to quantify life-history traits than traditional, macroscopic gonad examinations. Balon (1975) and Winemiller and Rose (1992) established that a classification system should also allow for intra-species comparisons for ecosystem based management and evolutionary life history comparison purposes. The use of a single histological classification system on multiple species provides a means to assess the reproductive biology of species that possess different reproductive strategies with variable life-history traits, and allows for comparisons between species.

Species of the family Serranidae: subfamily Epinephelinae (commonly called groupers and hinds) are heavily fished in the Caribbean. Despite their importance, large knowledge gaps exist about their reproductive biology. As with most marine fish, species-specific data is required for Epinephelinae because reproductive life-history traits are variable both within and between species (Sadovy 1996). In The Bahamas, fishery management and monitoring initiatives are focusing on securing consistent reproductive biology and landing data for commercially valuable Epinephelinae species.

Epinephelinae landings, especially *Epinephelus striatus* (Nassau grouper), and to a lesser, but considerable extent *E. guttatus* (red hind), and *Mycteroperca venenosa* (yellowfin grouper) constitute a major portion of fin-fish catches in The Bahamas (Cushion and Sealey 2007). To date, some reproductive life-history studies have been completed on *E. striatus* in The Bahamas (see Sadovy and Eklund 1999 for a review); while no formal studies on *E. guttatus* and *M. venenosa* have been conducted in The Bahamas.

This paper describes the effectiveness of a histology classification system for quantifying reproductive life-history traits and identifying the reproductive maturity stage of Epinephelinae species. The goal was to affirm that the proper criteria and diagnostics were incorporated into the system, so it could be applied to multiple Epinephelinae species that possess different reproductive strategies. The system was then used to determine the spawning seasonality for *E. guttatus, E. striatus, and M. venenosa* harvested in the central Bahamas. The classification system will form the basis for consistent long-term monitoring initiatives in The Bahamas and provide a means to evaluate temporal and spatial differences in Epinephelinae reproductive life-history traits that influence reproductive potential.

Material and Methods

A fishery-dependent monitoring project commenced in January 2007 at a major commercial fish market in New Providence (the most populated island), Nassau, Bahamas to acquire Epinephelinae landings, population, and reproductive biology data (Cushion and Sealey 2007). Data was obtained via monthly monitoring corresponding with the full moon phase (the spawning period of many Epinephelinae). A standard histological classification system was incorporated into the project to evaluate, compare and monitor reproductive traits among Epinephelinae species.

Monthly sampling was conducted at the market from January 2007-April 2008. Length, weight and gonad weight were measured and recorded for each fish. A section of each gonad was collected and preserved in 10% neutral buffered formalin. Gonad sections were later imbedded in paraffin, sectioned and stained using hemotoxylin and eosin following standard histological procedures (Fitzhugh et al. 1993). Gonad homogeneity tests to confirm that a subsample was representative of the entire gonad were previously performed for each species by Sadovy and Colin (1995) (*E. striatus*), Sadovy et al. (1994) (*E. guttatus*), and García-Cagide and Garcia (1996) (*M. venenosa*).

The reproductive biology classification system was adopted (with minor changes) from Lyon et al. (2008). Lyon et al. (2008) outlined a classification system based on previous studies including Moe (1969) and (Brown-Peterson et al. 2006). This system was adopted to classify Epinephelinae species for this study. Minor revisions were made to account for many Epinephelinae being protogynous species (thus having transitional gonads) and the common occurrence of "bisexual" gonads that contain both oogenic and spermatogenic tissue, but for which primary function as either male or female cannot be determined (Sadovy and Shapiro 1987).

Female and male fish were classified using diagnostic features to determine sexual maturity, the leading gamete stage (the most advanced oocyte or spermatogenic stage present), and whether oocytes were recently released (Table 1 a and b). The presence of vitellogenic oocytes indicates spawning will occur within days or weeks. Female spawning indicators are advanced vitellogenic oocytes (lipid and yolk coalescence) that represent the initiation of spawning and fully hydrated oocytes that are indicative of actively spawning fish. Recently spawned females were detected by the presence of post-ovulatory follicles. The end of the spawning season was determined by massive cell atresia (indeterminant spawners) or the lack of vitellogenic oocytes (determinate spawners). Male sexual maturity was indicated by initiation of spermatogenesis and formation of spermatocysts. Males are classified as spawning capable when spermatozoa were evident and filling sperm ducts and lobules.

Fish were classified as transitional if degenerating oogenic and proliferating spermatogenic tissue were present (Sadovy and Shapiro 1987). Fish were classified as bisexual if fairly equal amounts of oogenic and spermatogenic tissues were present, but no sexual function was determined (Sadovy and Colin 1995).

All histological slides for each species were analyzed and classified by two readers. Results were used to determine reproductive class. Also, the percentage of samples in each class was determined monthly and used to estimate the spawning seasonality for each focal species. Months were designated as spawning months if over 50% of the female samples for the month were classified as active or spawning and over 50% of the male samples were classified as spawning capable.

Results

The histological classification scheme modified and utilized for this study provided the appropriate criteria (Table 1) for designation of 96% of gonad samples

Table 1. A histological reproductive classification system and diagnostics for female, transitional, bisexual and male Epinephelinae.

Sex	Class	Diagnostics
Female	Immature, inactive	Primary growth oocytes only, no evidence of prior spawning. Chromatin nucleolus stage (small cells with large nucleus), and initial perinucleolar stage (larger oocytes). Well-organized gonad.
	Inactive, uncertain	Not capable of spawning in distant future & prior spawning unclear.
	Developing virgin, Developing	Cortical alveolar oocytes present. Prior spawning indicators confirm maturity (D). No spawning indicators (Dv).
	Active, mature	Vitellogenic oocytes present, will spawn within days or weeks.
	Spawning, hydrated	Early or late hydrated oocytes or post-ovulatory follicles present.
	Post-ovulatory, spent	End of spawning cycle, majority of oocytes (>50%) experiencing atresia. Post-ovulatory follicles may be present.
	Regressed, inactive, mature.	PG oocytes only, evidence of sexual maturity & recent spawn.
	Regressed, skipped, mature.	Sexually mature but will not spawn in current season, development ended prematurely.
Transitional	Sperm crypts proliferating throughout gonad. Gamete stages from primary spermatocyte through spermatid should be present. Remnant oocytes possibly undergoing atresia. Must possess evidence of degenerating oogenic and proliferating spermatogenic tissue. (Protogynous species only).	
Bisexual	Oogenic and spermatogenic tissues present, but neither is dominant or proliferating. No sexual function can be determined.	
Male	Immature, inactive	Includes males with spermatogonia (SGG) and no evidence of spermatogenesis (SG).
	Developing virgin (only gonorchoristic species)	Spermatogenesis begins; spermatocytes present & no prior indicators of maturity (Dv).
	Developing	Initiation of spermatogenesis and formation of spermatocysts (D).
	Spawning capable	Fish is reproductively active and capable of spawning. All stages of spermatogenesis may be present.
	Spent	Spermatogenesis is ceasing. Some residual spermatozoa present Spermatogonia proliferation and regeneration of germinal epithelium common in periphery of testis.
	Regressed, inactive, mature	Spermatogonia dominate; no active spermatogenesis. Continuous germinal epithelium throughout.

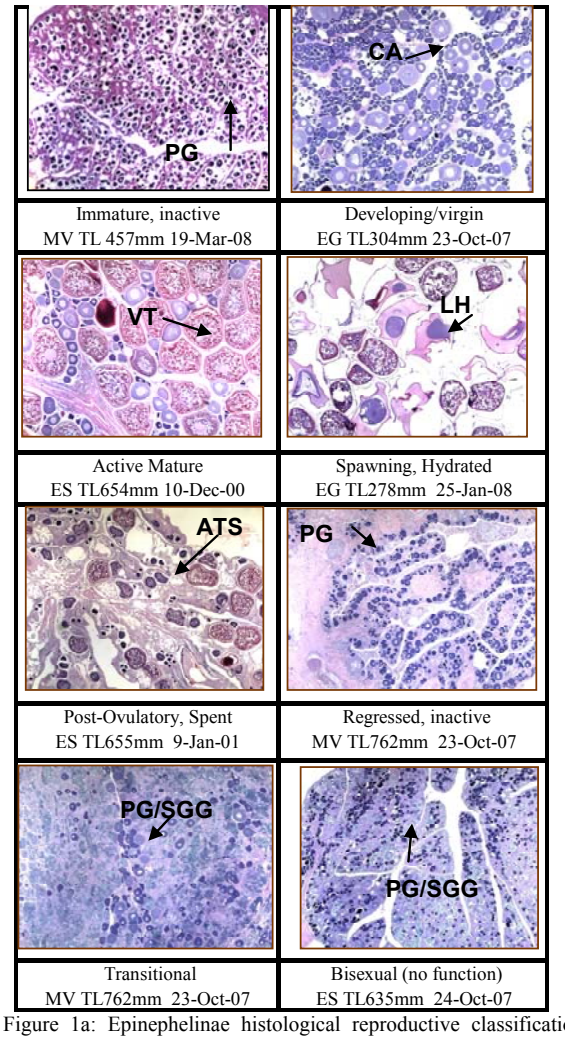

Figure 1a: Epinephelinae histological reproductive classification system for females, bisexual and transitional fish. Reproductive classes, diagnostic features, size and sample collection date are highlighted for female *E. striatus* (ES), *E. guttatus* (EG) and *M. venenosa* (MV). Primary growth (PG), cortical alveolar (CA), vitellogenic oocytes (VT), late hydrated (LH) and atresia (ATS) are highlighted.

(n=675) into a class (Fig. 1 a and b), all species combined. The results of this study corroborate previous reproductive biology studies on the focal species. Gamete production in *E. striatus* is indeterminate and the species is functionally gonochoristic (Sadovy and Colin 1995). *E. guttatus* is a protogynous hermaphrodite with determinate gamete production (Shapiro et al. 1993); while *M. venenosa* is a protogynous hermaphrodite with indeterminate gamete production (García-Cagide and Garcia 1996).

E. striatus samples were typically the most challenging to classify due to ~12% (26 out of 220) of all samples containing both inactive oogenic and spermatogenic tissue. The domination of oogenic or spermatigenic tissue was used to classify these fish, but 4% were classified as "bisexual" because no

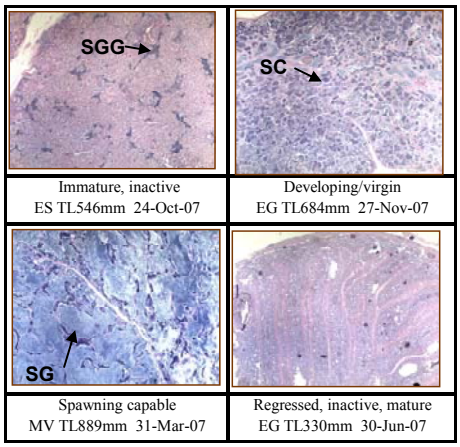

Figure 1b: Epinephelinae histological reproductive classification system for male fish. Reproductive classes, diagnostic features, size and sample collection date are highlighted for transitional, bisexual and male *E. striatus* (ES), *E. guttatus* (EG) and *M. venenosa* (MV). Spermatogonia (SGG), spermatogenesis (SG), and spermatocytes (SC) are highlighted. (Spent male not pictured.)

Table 2. Spawning seasonality for Bahamian Nassau grouper (*E. striatus*), Red hind (*E. guttatus*) and Yellowfin grouper (*M. venenosa*). Samples collected from January 2007- April 2008 in New Providence, corresponding to the full moon cycle. Spawning months were designated as so if over 50% of the female samples were classified as "Active" or "Spawning hydrated" and over 50% of the male samples were classified as "Spawning capable" (Table 1).

Species	**Spawning Years/ Months** *(Sample number in parentheses).*
E. striatus	November 2007 (21) January and February 2008 (27 and 23)
E. guttatus	February 2007 (17) January and February 2008 (22 and 30)
M. venenosa	March 2007* (12 and 28) March and April 2008 (28 and 34)
*Two sampling periods: one at the beginning and one at the end of the month, following the full moon schedule.	

sexual function could be determined. Also, eleven *E. striatus* samples (5%) were classified as "inactive, uncertain". For *E. guttatus* 12 out of the 200 hundred samples (6%) were classified as "inactive, uncertain". For *M. venenosa,* 15 out of the 175 samples (9%) were classified as "inactive, uncertain". For all species, the majority of samples classified "inactive, uncertain" were from the summer, non-spawning months.

Spawning seasonality for the three focal species was analyzed (Table 2). Over 50% of the male and female *E. striatus* samples in November 2007, January and February 2008 were in spawning condition (n=21, 27 and 23, respectively) (no samples were obtained in December 2007). For *E. guttatus*, over 50% of the male and female samples collected in February 2007, January and February 2008 were in spawning condition (n=17, 22 and 30, respectively). *M. venenosa* samples revealed their spawning season to be slightly later. Over 50% of the male and female samples collected during two sampling periods in March 2007 (one at the beginning and one at the end of the month, following the full moon schedule), and March and April 2008 (n=12, 28, 28 and 34, respectively) were in spawning condition. Additionally, 45% of the February samples were in spawning condition.

Discussion

The high percentage of classification for each focal species highlights the cross-utility of the classification system. The system allows for the requisite reproductive biology information to be quantified for Epinephelinae species in the Bahamas. The confirmation *E. striatus* as functionally gonochoristic was supported by the overlap of males and females in all size classes. This is unlike protogynous *E. guttatus* and *M. venenosa*, in that no males were found in the relatively smaller size classes and no females were found after a certain size (unpublished data). The percentage of *E. striatus* that were classified as bisexual, with no sexual function being determined was not unusual. Sadovy and Colin (1995) investigated the sexual development pattern of *E. striatus* and found four mature bisexual individuals and 23% of all samples were immature bisexuals. The classification of 4% of *E. striatus*, 6% of *E. guttatus* and 9% of *M. venenosa* as inactive, uncertain was also not uncommon. These samples were primarily from summer months when fish are not spawning. Inactive and regressed fish are the main classes during this time period and both are typified by compact gonads with primary growth oocytes. Thus, without sufficient evidence of prior spawning (e.g. old hydrated oocytes) it is not possible to confirm regression. Shapiro et al. (1993) investigated sex change and reproduction in *E. guttatus* and could not distinguish between inactive and late, regressed females.

Spawning seasonality for many Epinephelinae and other reef fish is a variable reproductive trait (within and between populations), especially for populations at different latitudes (Sadovy 1996). Spawning seasonality has previously been determined for *E. striatus* in the Bahamas. Colin (1992) found that the *E. striatus* populations off Long Island spawned during the full moon periods of December and January, possibly not during November and likely not during February. This study highlights that *E. striatus* spawning seasonality is slightly variable within the Bahamas. Spawning began in November 2007 and continued through February 2008. However, for *E. striatus,* spawning seasonality is strongly correlated with the lunar full moon as well as temperature, not the month *per se* (Sadovy and Eklund 1999). Colin

(1992) found *E. striatus* spawning occurred at water temperatures between 25.0-25.5°C. Thus, water temperature is likely a strong contributing factor for latitudinal and annual fluctuations in spawning seasonality.

This is the first documentation of spawning seasonality for *E. guttatus* and *M. venenosa* in The Bahamas. Shapiro et al. (1993) found a similar spawning seasonality for Puerto Rican *E. guttatus* populations. Using a gonadal size index and histology, spawning peaks were found in January and February. Meanwhile, *E. guttatus* spawning peaks much later in Bermuda occurring during the full moon periods from May to July (Luckhurst et al. 2004). It is noteworthy that *E. guttatus* spawning seasonality is not as tightly correlated to the full moon (Sadovy et al. 1994), as with *E. striatus* and *M. venenosa*. Thus the monthly, full-moon sampling regime did not likely capture all *E. guttatus* spawning activity in the Bahamas.

Small groups of *M. venenosa* are often associated with aggregations of *E. striatus* (e.g. Whaylen et al. 2004 (Cayman Islands), Nemeth et al. 2004 (USVI)). However, January and February are not the dominant spawning times for *M. venenosa*. Personal communications with fishermen in this study, in conjunction with gonad sampling, confirmed full moon periods during March and April as peak spawning months of *M. venenosa* in The Bahamas. A large proportion of the specimens were spawning capable in February, thus indicating the spawning period may commence in February. In Cuba, García-Cagide and Garcia (1996) found April and May to be the strongest spawning months for *M. venenosa* which is consistent with later spawning at more southerly latitudes.

Because reef fisheries in The Bahamas are multi-species, it is important to implement a system that can be applied to multiple species to ensure that consistent and reliable information is obtained. The fishery-dependent sampling protocol with a standard reproductive classification allowed for the collection and analysis of samples year round. This combination system will provide a means for long-term monitoring of Epinephelinae species to consistently assess reproductive life-history traits and the reproductive potential of populations.

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Spatio-temporal patterns of juvenile and adult abundance and biomass of reef fishes in the Sulu Sea, Philippines

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Abstract: Underwater fish visual census was undertaken to determine juvenile and adult abundance and biomass of commonly occurring reef fish taxa/species at several sites along the major marine corridors in the Sulu Sea. Presence/absence, abundance, and biomass of taxa/species in both stages showed varying patterns within and among sites. Variation in adult abundance and biomass of some species suggests decreasing similarities with increasing distance at some sites while other species showed contrasting patterns with distant sites exhibiting similarities. For juvenile abundance, patterns were similar with those of the adults. Significant correlation between juvenile and adult abundance was observed at some sites. The match and mismatch of spatial patterns of distribution of adults and juveniles of reef-associated fishes are influenced by two major factors. Local water circulation patterns at the different corridors, which potentially disperse egg and larvae within and between corridors shows changing connectivity potential of fish populations. Relatively high species diversity of juvenile and adult fish was observed in areas of high entrainment. On the other hand, disturbance and stresses such as over-fishing and habitat degradation will increase mortality in fish populations at varying stages of their life history and, therefore, reduces the connectivity potential in a range of spatial scales in the Sulu Seascape.

Keywords: reef fish abundance and biomass, entrainment and habitat degradation, spatial and temporal patterns

Introduction

The marine corridors in the Sulu Sea have been proposed to be strategic marine priority conservation areas that help provide the resiliency in the Sulu Sea (Ong et al. 2002). These areas are found at the heart of the Coral Triangle, which has been recognized as the center of highest marine biodiversity (Carpenter and Springer 2005). Regular monitoring of adult and juvenile reef fish abundances serve as crucial proxies to reef conditions and help understand the major determinants of the population structure of coral reef fishes (Booth and Beretta, 2004).

Observations of the changes in reef benthos and community structure of associated fish dovetail with other factors such as hydrography (Cowen 2002) and larval biology (Leis and McCormick 2002) to fully understand the connectivity of reef populations (Cowen et al. 2006). This study presents the dynamics of the reef fish community structure in the marine corridors of the Sulu Sea.

Materials and Methods

A total of 44 transects from five municipality sites covering the three marine corridors in the Sulu Sea were sampled (Mabini and Verde in the Verde Island Passage; Balabac in the Balabac Strait; Cagayancillo and Tubbataha reefs in the Cagayan Ridge) (Fig.1). Survey months were made during the transition periods of the Northeast monsoon (October 2006) and Southwest monsoon (April-May 2006 and 2007). Fish Visual Census (FVC) was used to determine fish assemblages and abundance of adults and juveniles (English et al. 1997). Fish were identified if possible at the species level, their numbers and sizes estimated within an area of $500m^2$ (adult) and $50m^2$ (juvenile) per transect.

Multivariate analyses of spatial pattern of adult and juvenile fish correlation with forcing factors were performed using the ordination technique, non-metric Multidimensional Scaling (nMDS). In addition, Analysis of Similarities (ANOSIM) was carried out to determine significance of generated patterns (PRIMER 6 ver. 6.1.6). This approach examines factors that influence the spatial and temporal patterns

of the reef associated fish communities. From the patterns we can infer insights that will facilitate marine biodiversity conservation measures to reduce the threats on reef health and help in the design of MPA networks.

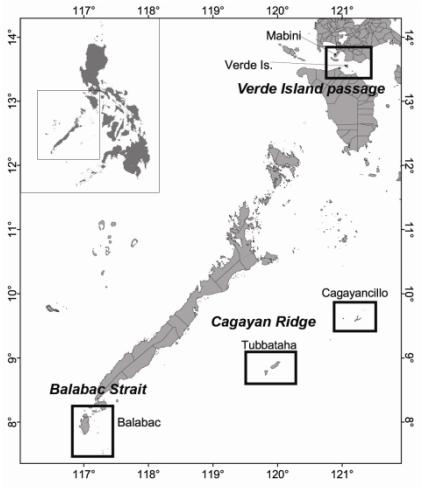

Figure 1. Map of study sites within the three major marine corridors (i.e., *Verde Island Passage, Balabac Strait and Cagayan Ridge* includes Tubbataha and Cagayancillo.

Results

Adult abundance and biomass

Presence/absence, abundance and biomass of taxa/species at adult stages showed varying patterns within and among the municipality sites in the three corridors. Non-metric MDS analysis for abundance showed clear patterns only in between-site category (Fig. 2a). For biomass, ordination analysis provided similar patterns as with abundance. Analysis of Similarity (ANOSIM) of abundance and biomass revealed that factor Site (municipality) was more important than Time (season) (Table 1) suggesting that site-specific attributes are more important forcing factors upon the fish community structure.

Pair-wise comparison for differences in abundance and biomass between municipalities showed large variation between Balabac and Tubbataha (Table 2) especially for the abundances of families Apogonidae, Pomacentridae, Caesionidae Labridae, Lutjanidae, Anthiinae and Acanthuridae. There were more apogonids and labrids (Cheilininae) at Balabac than at Tubbataha but more caesionids, pomacentrids, acanthurids, and lutjanids at the latter than at the former. Between Tubbataha and Mabini/Verde sites, there were more caesionids, acanthurids, lutjanids and serranids at Tubbataha than at Mabini/Verde. Some sites showed increasing similarities with decreasing distance (e.g. adjacent sites Cagayancillo-Tubbataha versus distant sites Cagayancillo-Mabini/Verde) based on the abundances of dominant taxa such as caesionids, acanthurids, lutjanids and serranids. However, distant sites such as Balabac and Mabini/Verde also showed similarities based on the abundances of pomacentrids (*Acanthochromis polyacanthus*) and anthiinids (*Pseudanthias huchtii*). Following Tubbataha in reef fish biomass is Cagayancillo, Mabini, Verde and Balabac. Consistent peaks were observed for Tubbataha for the three intermonsoon seasons, while Mabini was observed to be highest in October. Palawan sites were recorded with highest biomass in the April-May transition periods. In addition, its relatively isolated location in the Sulu Sea (island mass effect; Hammer and Hairy 1981, AMERCO and Andrews 1989) affords the Cagayancillo reefs the high abundance observed.

Temporal pattern in abundance did not differ (April-May 2006 and April-May 2007; $R=0.107$, $p<0.01$) whereas biomass did so slightly. This was attributed to an increase in target species at Verde from 2006 to 2007 and a decrease at Tubbataha from 2006 to 2007.

Table 1. ANOSIM of adult fish abundance and biomass for the five municipalities surveyed over three monitoring periods. Factor with highest R-value contributes more to the community structure.

Factors	R-value	Sig. Level (%)
Abundance		
Time	0.101	0.4^{**}
Site	0.369	0.1^{**}
Biomass		
Time	0.046	6.7^{ns}
Site	0.507	0.1^{**}

The abundant presence of species belonging to the large, top trophic groups (Lutjanidae, Serranidae, Acanthuridae, and Scaridae) resulted in higher biomass in Tubbataha. This was not surprising since Tubbataha is a no-take National Marine Park with strict enforcement resulting in a considerable number of large, top predatory fish whereas Balabac and Mabini/Verde sites are under various levels of exploitation with few smaller marine reserves.

Table 2. Pair-wise test for difference in reef fish abundance (Ab) and biomass (Bm) between groups of municipalities surveyed for the three monitoring periods. R-values approaching '1' indicate large differences.

Groups	R-value		Sig. level (%)	
	Ab	Bm	Ab	Bm
Bal, Mab	0.32	0.34	10.7	9.5
Bal, Ver	0.26	0.43	0.3	0.1
Bal, Cag	0.39	0.41	0.1	0.1
Bal, Tub	**0.75**	**0.91**	**0.1**	**0.1**
Mab, Ver	0.13	0.50	20.6	0.2
Mab, Cag	0.26	0.29	1.4	0.2
Mab, Tub	**0.51**	**0.84**	**0.1**	**0.1**
Ver, Cag	0.35	0.48	0.1	0.1
Ver, Tub	**0.50**	**0.78**	**0.1**	**0.1**
Cag, Tub	0.17	0.50	0.7	0.1

Juvenile abundance

Density (no. per area) of juveniles showed different seasonal pattern compared to adults. Tubbataha, Cagayancillo and Verde peaked in juvenile abundance

during October while Balabac and Mabini peaked during April-May (Fig.4). Tubbataha and Cagayancillo recorded very high juvenile densities, about 6 to 9 times more than the other municipalities surveyed, coincident also with high abundance in adult fish. nMDS analysis for juveniles showed less distinct patterns than that of the adult fish (Fig.2b). In order to identify which taxonomic group of fish drives the variation in abundance, BVSTEP analysis was undertaken. Families Apogonidae and Cirrhitidae were the important fish groups for Balabac and Cagayancillo, while it was Anthiinae for Tubbataha, Verde and Cagayancillo. Gobiidae, on the other hand, was prominent in Cagayancillo and Verde whereas Labridae subfamily Bodianinae was the significant fish group in Cagayancillo and Tubbataha. ANOSIM revealed that both factors Site and Time had very low R values (0.183 and 0.094, respectively) which suggest that the temporal patterns of juvenile abundance are similar among sites. Nonetheless, there appears a difference in the level of abundance between years and seasons at some sites (Fig. 4). There were more juveniles of family Pomacentridae and Anthiinae in April and October 2006 at Tubbataha and Cagayancillo. There was generally higher abundance of juveniles of family Pomacentridae and Anthiinae in 2006 than in 2007 at Tubbataha, Cagayancillo and Verde. These results indicate that recruitment may be seasonal at least at Tubbataha, Cagayancillo and Verde (Fig. 4).

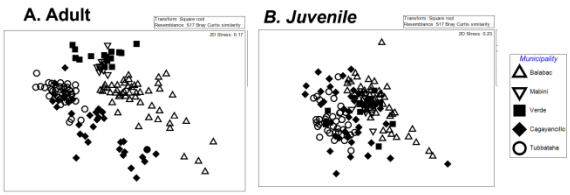

Figure 2. Spatial pattern of (a) adult (b) juveniles fish abundance in 44 transect sites in three monitoring seasons.

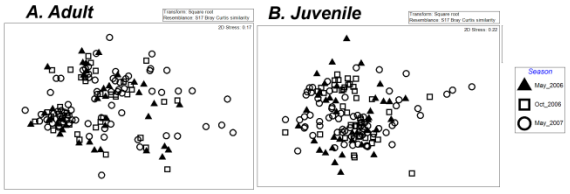

Figure 3. Temporal pattern (a) adult and (b) juvenile abundance in 44 transect sites in three monitoring seasons.

MDS analysis also showed no clear patterns on juvenile abundance (Fig. 3b).

Correlation between adult and juvenile reef fish abundance among the five municipalities for the three sampling seasons revealed low but significant correlations overall ($r=0.4$, $p<0.01$) and per sampling season (May 2006: $r=0.4$, $p<0.01$, Oct. 2006: $r=0.5$, $p<0.01$, and May 2007: $r=0.5$, $p<0.01$). These similarities in abundance trends in adults and juveniles at specific sites can be drawn from patterns in community structure exhibited by dominant families. An example in the study would be Anthiinae identified in the BVSTEP analysis. There was good correlation between juveniles and adults of Anthiinae in Balabac ($r=0.73$, $p<0.05$) and in Verde ($r=0.78$, $p<0.05$). Apogonidae, on the other hand, was highly correlated in Balabac ($r=0.69$, $p<0.05$) but not so much in Cagayancillo ($r=0.14$, $p>0.05$) wherein Cirrhitidae correlated well ($r=0.62$, $p<0.05$). Cirrhitids also significantly showed good correlation in Tubbataha ($r=0.42$, $p<0.05$) together with Bodianinae ($r=0.42$, $p<0.05$). However, this phenomenon is not common in many reef fish families (e.g. Srinivasan and Jones 2006).

Discussion

Some patterns in adults showed decreasing similarity with increasing distance as exemplified by the distant Tubbataha-Balabac sites and proximal Tubbataha-Cagayancillo sites. This pattern could be influenced by processes driving larval dispersal (e.g. Cowen et al., 2000) and thus provides an explanation of the present fish community structure (e.g. Sale and Kritzer, 2003). Beldia et al. (this symposium) have provided data on larval distribution together with oceanographic profiles of the study sites and clearly showed potential larval sources and sinks. However, there were contrasting patterns which showed similarity even in distant sites (e.g. Balabac-Verde). This scenario is completely different and hardly invokes ecological processes as to explain the community structure. Stressors such as fishing and habitat degradation also potentially influence the variation in the patterns. There were differences in the occurrence of target species such as caesionids, acanthurids, labrids and lutjanids among the sites.

Juvenile abundance showed slightly different temporal pattern compared to adult abundance. Recruitment may be seasonal at least in Tubbataha, Cagayancillo and Verde, which peak during October month. However, temporal pattern may be site specific and seasonal trend may not always be consistent between sites (Sale et al., 1984) and seasons (Arceo 2004, Booth and Beretta 2004, Srinivasan and Jones 2006). Among the municipalities, Tubbataha and Cagayancillo recorded very high juvenile abundance than the other sites surveyed, coincident also with high abundance in adult fish (Srinivasan and Jones 2006). This suggests that recruitment is high in Cagayancillo and Tubbataha sites or it has dual functionality of being a source and sink area. Furthermore, Cagayancillo sites

might also act as a sink, receiving larvae from areas farther north such as the Calamianes group of Islands, Cuyo, Agutaya and Apo Reefs via the prevailing water currents during the Northeast monsoon, and receiving larvae from Tubbataha during Southwest monsoon (Fig. 5). Mabini/Verde, on the other hand, may be receiving larvae from Mindoro and Tayabas Bay. This however still remains to be confirmed.

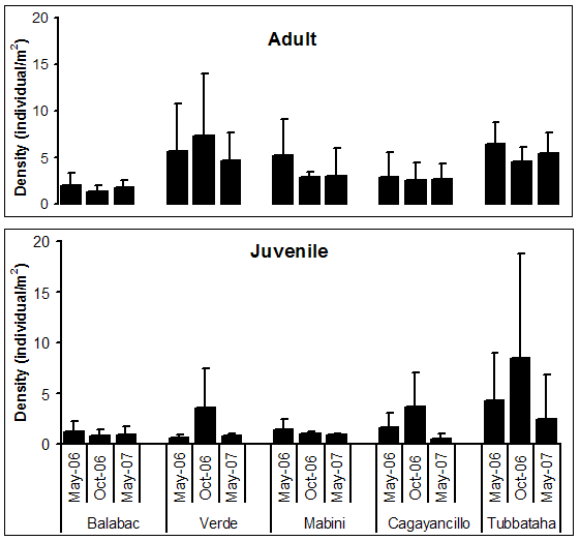

Figure 4. Density of adult and juvenile fish. Recruitment may be seasonal at least in Tubbataha, Cagayancillo and Verde, which peak during October.

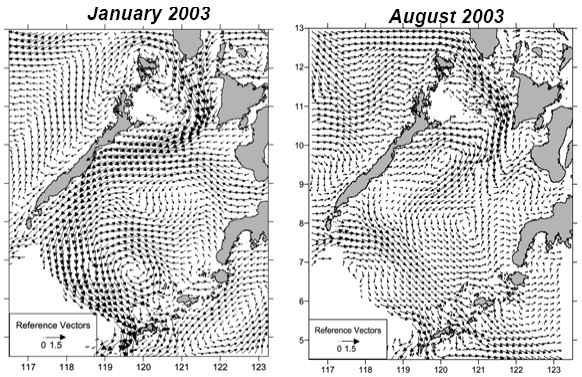

Figure 5. Current patterns in Sulu Sea derived from the Pacific HYCOM (http://hycom.rsmas.miami.edu/) used to force dispersal model for the entire basin (Sulu-Sulawesi Seascape).

These matching and mismatching of spatial patterns of distributions of the various life stages of benthic reef-associated fishes in Sulu Sea are influenced by two major environmental factors. One is the local water circulation pattern (Sale et al., 1984, 2005, Bode et al., 2006) at the different corridors in the Sulu Seascape, which potentially disperses egg and larvae within and between corridors, implicating shifts in connectivity potential of fish populations. There is a concordance on the diversity of juvenile fish species in areas of high entrainment (Carassou and Ponton, 2007) and with adult fish species. The second major factor is disturbance and/or stresses such as over-fishing and habitat degradation. Mortality in fish populations at varying stages of their life history will therefore reduce the connectivity potential in a range of spatial scales in the Sulu Sea (e.g. Sale et al., 1984, Sale and Kritzer, 2003).

Conclusions

Abundances of adult and juvenile fishes were high in Tubbataha and Cagayancillo suggesting that recruitment, survivorship and growth rates in these areas are also high which may serve both as a source and as a sink.

Adult fish had clearer spatial pattern than juveniles. ANOSIM revealed significant differences vis-à-vis their location among sites indicating site as a factor affecting fish community structure. However, juvenile had clearer patterns in terms of temporal pattern than former.

Spatial distribution patterns were influenced by (a) local water circulation patterns which potentially influence the connectivity of fish populations and (b) disturbance and/or stresses (i.e., over-fishing and habitat degradation) which increase the mortality of fish populations at varying life stages and therefore affect their survivorship and reproductive outputs at each corridor.

Results from this study fill critical gaps in developing scenarios on connectivity and their implications to management and biodiversity conservation. These areas would be important in the design of MPA networks and in the improvement of critical management gaps such as reducing threats through integrated coastal management, enhancing survivorship, strengthening enforcement and education about reducing threats and disturbances.

Acknowledgement

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Size spectra analysis as a tool to examine management effectiveness of marine protected areas

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Abstract. Following extensive community consultation and resource assessments the allowable uses within Karimunjawa National Park (KNP), Indonesia, were re-zoned in 2005. The aim was to improve the health of coral reefs and increase the biomass of reef fish. We evaluated the effectiveness of zoning by comparing the abundance and size of reef fishes among no-take zones (entry prohibited), no-take zones (entry permitted) and fishing zones. The census included all reef fish species and each fish was placed into size class categories of 5cm increments from 0 to 40+ cm. Biomass was calculated using established weight-and-length relationships. The biomass of large fishes was generally higher in no take zones (mean ± S E: 536 ± 113 kg ha^{-1}) than in no-take zones (entry permitted) (353 ± 63 kg ha^{-1}) and fishing zones (301 ± 39 kg ha^{-1}), although the effect was not significant. Size spectra analyses showed that reef fish structure did vary among management zones with reef fishes in size class of 15-20cm and 35-40cm most abundant in the no-take zone (entry prohibited). Fish in the no-take zone (entry prohibited) were mostly of target species, including fusiliers, emperors, snappers and groupers. In contrast, small (5-10cm) non-target species, mainly wrasses and cardinal fishes, were more abundant in the fishing zones. The higher biomass of large sized fishes inside no-take zones (entry prohibited) is most likely because fishing pressure has been reduced. Although enforcement of fishing regulations inside the park remains inadequate, the results suggest that fishing restrictions are receiving support among fishers and if support continues fish stocks in all zones may increase.

Key words : Karimunjawa National Park, effectiveness management, size spectra, biomass.

Introduction

Karimunjawa National Park is one of eight national marine protected areas in Indonesia and is situated 120 km north of Semarang, Central Java, Indonesia (Fig. 1). Based on Indonesia law No. 5/1990 regarding the conservation of natural resources and ecosystems, the national park is managed by a zoning system. A rezoning process was conducted from 2003 to 2005 as part of an effort to design an effective management system by the National Park Authority with the aim of improving health of coral reefs and to increase the biomass of reef fish. We evaluated the effectiveness of zoning by comparing the abundance and size of reef fishes among no-take zones (entry prohibited), no-take zones (entry permitted), and fishing zones.

Material and Methods

The fish community was sampled using visual census methods recording all fish to species level, except for gobies (Gobiidae), blennies (Bleniidae), and triplefins (Tripterygidae). Fish biomass was calculated using total length (cm) of fish and converted into weight (kg) using length-weight relationships (Froese and Pauly 2000).

Information on fishing grounds, number of fishers, and type of gears were collected during interviews with fishers in Karimunjawa National Park between March 2005 and February 2006.

Two-way ANOVA were used to test for differences in fish biomass between management zones and size classes and between, management zones and trophic groups. Data were tested for normality and log transformed prior to analysis.

Results and Discussion

Reef fish biomass in no take zones (entry prohibited) was significantly higher than in no take zones (entry permitted) and fishing zones. Size structure also differed between zones with high biomass of fish in size classes from 15 cm to 40 cm in no take zone (entry prohibited) than the other 2 zones. (Fig. 2).

Reef fishes in the no take zone (entry prohibited) and no take zone (entry permitted) had higher size

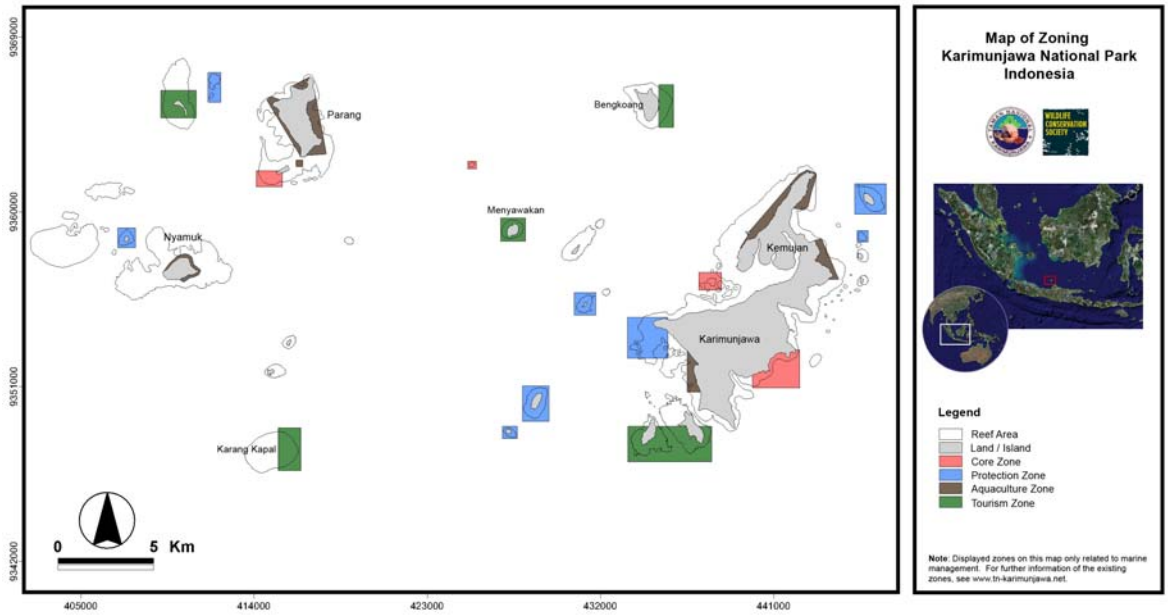

Figure 1. Map of Karimunjawa National Park, Central Java Indonesia.

classes, from 15-20 cm to >40 cm. Most fish in these size classes were fusiliers (Caesionidae), snappers (Lutjanidae), groupers (Serranidae), and emperor (Lethrinidae), which are target species for fisheries in Karimunjawa. The majority of fishes in fishing zones size class consisted of wrasses (Labridae) and cardinal fishes (Apogonidae, Fig. 3).

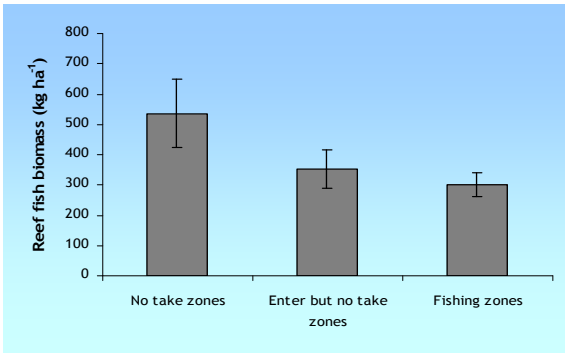

Figure 2. Reef fish biomass in 3 management zones.

Table 1. Two-way ANOVA on fish biomass (kg ha^{-1}) management zones and size structure. Data was log transformed prior to analysis.

Source	df	MS	F	P
Biomass				
Management Zones	4	4.801	3.380	0.010
Size Structure	8	73.501	51.738	0.001
Management Zones x Size Structure	32	1.260	0.887	0.647
Error	342	1.421		

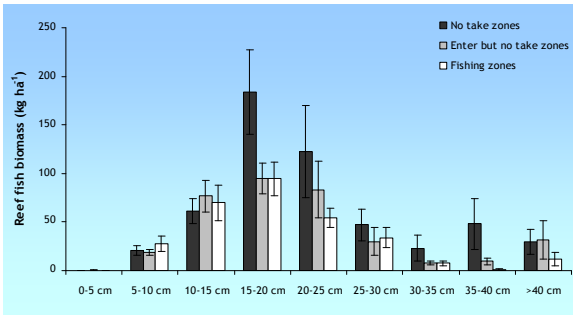

Figure 3. Reef fish biomass (kg ha^{-1}) by size structure in 3 management zones.

Signicantly high biomass of planktivore and carnivore were found in the no take zones (entry prohibited) than in the other zones (Table 2). Higher biomass of planktivore and carnivore were found in the no-take zone (entry prohibited) whereas a higher biomass of herbivores was found in fishing zones (Fig. 4).

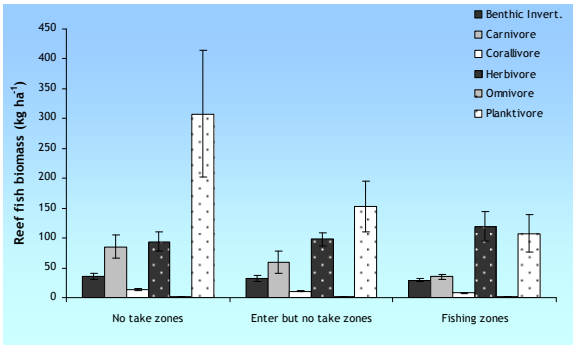

Figure 4. Reef fish biomass (kg ha^{-1}) by trophic groups in 3 management zones.

Table 2. Two-way ANOVA of on fish biomass (kg ha^{-1}) management zones and trophic groups. Data was log transformed prior to analysis.

Source	df	MS	F	P
Biomass				
Management Zones	4	0.904	1.759	0.138
Trophic Groups	5	67.807	132.007	0.001
Management Zones x Trophic Groups	20	0.803	1.564	0.064
Error	21	0.514		

Fishing Intensity

The numbers of fishing trips in each zone revealed that the fishing pressure in the no take zone (entry prohibited) and no take zone (entry permitted) were lower than in the fishing zone (Figure 5).

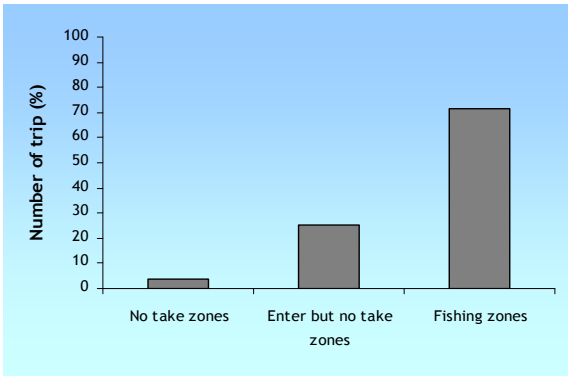

Figure 5. Fishing pressure in 3 management zones.

Conclusions

- Reef fish biomass in the no take zones (entry prohibited) was higher than in no take zones (entry permitted) and the fishing zones.
- Medium and large size classes of target species were most abundant in the no-take zone (entry prohibited).
- Reef fish condition will be improved if all stakeholders and fishers have awareness of sustainable fisheries practices to achieve better management in Karimunjawa National Park.

Acknowledgement

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The Relevance of Traditional Ecological Knowledge for Modern Management of Coral Reef Fisheries in Melanesia

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Abstract - Traditional ecological knowledge (TEK) has received great attention in respect to coral reef associated fisheries as a way to adapt modern management strategies to local environmental and cultural conditions. We analysed the social and cultural roles of TEK for resource management in traditional Melanesian communities in New Caledonia. A multidisciplinary survey of customary marine tenure and fishing regulations on Ouvéa, a raised limestone island in New Caledonia, was carried out in 2006. Informants from the main chiefdoms and clans were questioned about past and present fishing activities, maritime territory rights, taboo areas and place names, customary authority, socio-cultural practices and belief related to marine resources, and vernacular knowledge and taxonomy of marine organisms. Results showed that customary fishing rules were primarily related to cultural events and social organization rather than to ecological patterns or economic interests. The relationships between TEK, population needs and uses of the environment were still strong, but have changed since the 1860s. An unquantifiable loss of indigenous knowledge has also occurred. Nowadays, to satisfy food and economic needs, modern users often abandon TEK and behave in relation to their own individual economic perceptions and needs. Better consideration of social and cultural aspects in resource management issues may therefore directly help to increase awareness of resource depletion and biodiversity loss as a basis for achieving long-term ecosystem and economic sustainability in Melanesian islands.

Key words: coral reef, fisheries management, traditional ecological knowledge, New Caledonia, South Pacific

Introduction

Marine traditional ecological knowledge (TEK) is part of the larger body of indigenous knowledge. It is defined as a complex of knowledge of sea (i.e. reefs, lagoons and target species), livelihood activities (fishing techniques and practices, management rules), and related magic, beliefs and legends of social and cultural values (Berkes and Folke 1998). This knowledge constitutes a user-based accumulation of experience and observations that has been improved and transmitted orally through generations (Ruddle 1993; Turner and Berkes 2006a).

A large part of the literature about marine TEK has addressed the local knowledge of the environment or the taxonomy, the biology and the ecology of marine organisms (Aswani and Hamilton 2004; Lauer and Aswani 2008; Fraser et al.. 2006) as well as the sustainability of indigenous practices (Johannes 2002; Lobes and Berkes 2004). Zooarchaeological evidence has also shown that not all practices were conservative and that early human settlement in many remote Oceanic islands led to sharp depletions of virgin terrestrial and marine resources, such as birds and shellfishes (Kirch and Hunt 1997; Steadman 1995). One the other hand, there is increasing evidence that TEK can improve the understanding of resource use patterns and the monitoring and the adaptive management of coastal fisheries (Danielsen et al. 2005; Turner and Berkes 2006b). This case study of Ouvéa Island (New Caledonia), a raised limestone island with Melanesian and Polynesian communities, is an empirical evaluation of the relationships between TEK and the modern management of coral reef fisheries. Here we provide an interpretation of the social and cultural significance of TEK for indigenous populations and the roles that it could play in the sustainability of contemporary fisheries in Melanesia.

Materiel and methods

The functions of TEK within the Melanesian socio-political organization

In the traditional Melanesian cultural system, intrinsic interactions between humans and nature gave TEK a key role in regulating subsistence fisheries (Leblic 1989): the fishing system was driven by a dynamic linkage between community needs, TEK and uses

(Fig. 1a). Traditional socio-political organization resulted in a complex management system of fishing activities that were broadly controlled by TEK, but did not fully succeed in preventing overexploitation. The co-evolution of TEK with the surrounding environment was necessary to respond to both gradual and rapid change and to meet basic food and other subsistence needs. These needs were also linked to an array of social and cultural factors that determined formal and informal rules, codes of conduct, stories, and local taxonomies, among others. Particularly important factors related to TEK included the legitimacy of knowledge holders and territory owners, competition for leadership, the differentiation of the socio-political roles among people, and clan identity within the Melanesian society (Bensa 1995; Guiart 1992).

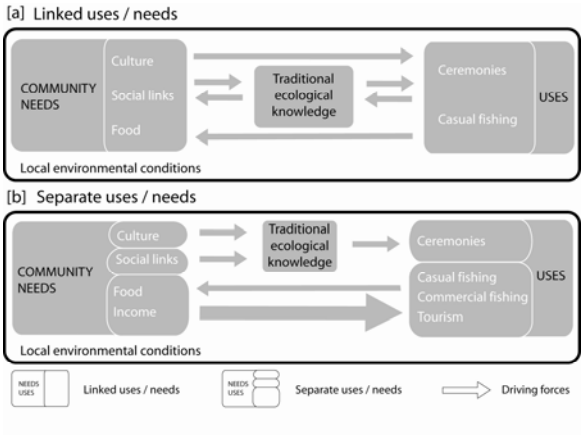

Figure 1. Relationships between traditional ecological knowledge, needs, and uses of marine resources in the traditional Melanesian cultural system [a] and in modern fisheries [b].

These roles were usually linked to the skills and totems of many successive migrants from different origins. As in many other Pacific islands, over the last few centuries, several waves of settlement by groups of both Melanesian and Polynesian origin are documented for Ouvéa since the first occupants arrived from Vanuatu or the Salomon Islands 4000 years ago (Guiart 1952; Sand 1998). These include settlers from the nearby islands of Lifou and Grande Terre (the main island of New Caledonia) and from Polynesia (Samoa, Wallis or West Uvea, and Tonga). The latter Polynesian groups settled in the northern and southern parts of the island in the 18th century. The body of Ouvéa TEK has, thus, been shaped by the importation, aggregation, synthesis and transformation of different sources of knowledge, including the Melanesian (*Iaai*) and Polynesian (*Faga Uvea*) languages or techniques (e.g, the distinctive construction of wooden canoes or sailing boats).

Study site and survey design

Ouvéa is a crescent-shaped raised limestone atoll-like island with a total land area of about 50 km^2, which extends some 51 km from the northwest to the southwest and is about 7 km wide at its widest point (Fig. 2). The island encircles a large 836 km^2 lagoon with depths up to 25 m deep that is enclosed by barrier reefs and islets along its northern and the southern shores. The inner lagoon coast is lined with an almost continuous 30 km-long white sand beach, whereas the outer Ocean side coast has raised limestone terraces and fringing coral reefs along most of its extent.

The local population (4400 inhabitants 97 % of whom are Melanesian) is distributed in 20 traditional tribes, who have *de facto* collective control over Ouvéa waters. This customary system is contradictory to New Caledonia law that stipulates that lagoon and shoreline are public property and thus open to all.

Available quantitative data on fish resources and catches are included in the analysis. The annual catch of fish was estimated to be 200 t and was mainly composed of Lethrinidae and Serranidae (Léopold et al.. 2004). More than 90 % of this catch was harvested in the lagoon area (about 95 % of the fishing grounds). About 15 % was sold on the local market; commercial fish exports were very low. This low fishing pressure has markedly preserved the fish biomass of the island (Kulbicki et al. 1995).

Using open-ended and qualitative interviews, we conducted a multidisciplinary survey in the northern and the southern Districts in 2006 to explore the traditional relationships between Kanak residents and their reefs and lagoon. The methodology consisted of interviews of all customary leaders of the different clans and chiefdoms of both Districts, who were questioned about social and cultural practises and stories related to the marine environment, customary authorities, local knowledge on marine life, fishing activities, territory rights, taboo areas and place names. High resolution aerial photography was used to localize sites and related data when appropriate.

Results

Fifty-one interviews of knowledgeable people were completed. We found that resident fishers have open access to the entire lagoon area, whereas outsiders coming from neighbouring islands for recreational activities or professional fishing are vigorously prosecuted.

All emerged areas and adjacent reefs are owned by the first settlers who have sometimes granted their guardianship to another social group. Interestingly, there are no local place names or traditional uses mentioned for the lagoon area, which suggests that there has been little need to depend on these

resources and areas for traditional purposes. Well-defined clan or tribal fishing grounds or territories are defined in reef and lagoon areas of the Northern and Southern Pleiads (about 40 km^2), and along the windward fringing reef (about 4 km^2) (Fig. 3). These are identified by vernacular place names of reefs, coral heads, passes, beaches, cliffs, etc. that remind people of the itineraries and myths of clans' ancestors. Species and gear restrictions are applied in a number of taboo sites where stories have recorded mythic relationships between people and marine animals (sharks, the humphead wrasse *Cheilinus undulatus*, morays, sea turtles, etc.). Specific customary rules also apply to sea turtles and large jacks (e.g. *Caranx ignobilis*) that are killed and shared according to ritual ceremonies.

No no-take zones were recorded but two types of reserved areas are present (Fig. 3):

i) Four marine areas are controlled as customary temporary reserves: 1) Lékine-Faiava reserve in the east, which extends into a shallow lagoon marked by strong tidal influence; 2) Mouli reserve in the southeast and 3) Teuta reserve located inside the lagoon alongside a coral cliff and a sandy beach respectively; and 4) Ognat reserve, which extends over the windward fringing reef. Their surface areas range between 0.2 and 1.5 km² and total about 2.5 km². Openings of the reserves to fishing are not implemented for a defined period but rather depend on social agendas and resource availability that may not be predictable. Interestingly, the protection status inside all taboo areas encompasses the physical environment and protects the reef habitats from direct human disturbances (e.g. wharf building).

Castnet and handline subsistence fishing is allowed in the four taboo sites because, according to local perceptions, such activities do not impact as negatively on fish resources as more effective methods (e.g. spearfishing, poisoning, and gillnetting). Significant fishing effort is only deployed during collective fishing trips to celebrate major social events (e.g. marriages, funerals, church ceremonies, etc.), which occur once to several times a year. Successful fishing is associated with abundant catches over a period of only a few hours, during when numerous fishers use nets hundreds of meters in length. Schooling species, such as the Indian mackerel (*Rastrelliger kanagurta*) and mullets (Mugilidae) are specifically targeted in Mouli and Teuta reserves respectively, whereas mainly reef species (Acanthuridae, Scaridae, Siganidae, etc.) are targeted in the two other taboo areas. The catches occasionally exceed a ton per day.

The main reason for the establishment of temporary reserves is to maximize catches during collective fishing trips. However this utilitarian pursuit for food should not hide its fundamental social and cultural importance. Such events remind fishers of their distinct customary roles based on traditional sociopolitical organization and their TEK. Such fishing activities are deeply embedded in social arenas where gifts of fish are used to strengthen and legitimize the hierarchical structure of the chiefdoms. Consequently traditional fishing rules may not survive the weakening of the social structure and the loss of TEK. For instance the ban on mullet net fishing in the Teuta taboo area is linked to the tribe's allegiance to the Weneki chiefdom, which is no longer enforced since the custodians have contested the authority of the land and marine resource owners on the site. As a result, the mullet fishery was recently opened to commercial harvest.

Similar clan-based political strategies and territory rights drive other collective fishing events on the windward fringing reef of the main island. The same net fishing techniques are used to target reef species, particularly the forktail rabbitfish *Siganus argenteus*.

ii) The Kanak of Mouli and Saint-Joseph Districts are historically and culturally attached to the Southern and Northern Pleiads respectively, where there are traditional plantations on all islets, and two of them (namely Bagaat and Unyee) were inhabited until the early 1900s. The nearby reefs, passes, and lagoons are also reserved for the collective benefit of the local residents as a food source though fishing rights are not exclusive. Fishers from Fayaoue District are allowed to use trawling and bottom lines in the areas for subsistence and commercial activities, and spear guns once they have formally gained the land owners' or the custodians' permission.

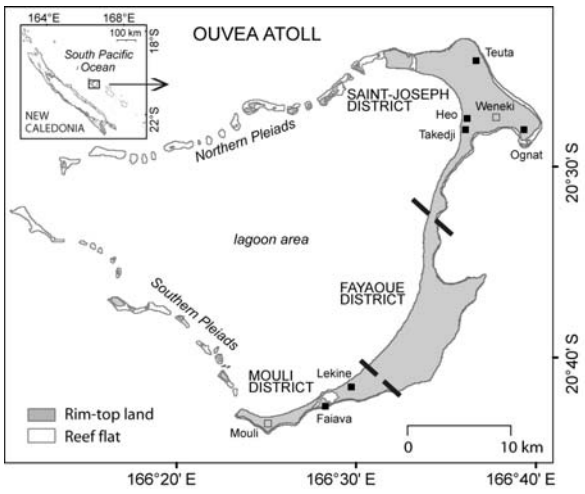

Figure 2. Ouvéa atoll (New Caledonia, South Pacific). Squares: Melanesian tribes in the survey area (Mouli and Saint-Joseph Districts). Unfilled squared: main chiefdom of both Districts.

Spear fishing was recently banned in the southern coastal waters to prevent shark attacks near the main tribal villages. This is the only locally-based decision that is not linked to Ouvéa social and cultural organization.

Discussion

Our results show that on Ouvéa fishers rarely used TEK and referred to recent experiences of the use of modern fishing gear when looking for food and income. European settlement and economic development have indeed changed the relationships between Melanesian people and their marine environment due to technical, geographical, socio-political and educational developments since the 1860s (Izoulet 2005). Similar trends occur in traditional societies in other Pacific Islands and countries (Murray et al.. 2006; Wittersheim 2006; Sillitoe 2000; Johannes 1978). On Ouvéa boats with outboard engines have given fishers access to new and more-distant fishing grounds inside the lagoon area rather than having to depend nearby surrounding fringing reefs and beaches off the main island and the Pleiads (Léopold et al. 2004), where traditional territories are located. More efficient fishing gear (e.g. nylon nets, spear guns) has also made fishing less uncertain, even for inexperienced fishers. More globally, progressive changes in population needs have finally marginalized fisheries-related TEK (Fig. 1b).

We found that TEK still has an important role in the Melanesian sociopolitical structures on Ouvéa. It now mainly addresses the social and cultural need to maintain traditional links between people, clans and chiefdoms rather than to protect the fishing economy or the environment *per se* (Fig. 1b). It is therefore a major part of the Kanak cultural heritage, as are marine resources and landscapes. Importantly sociocultural needs seem to be the driving force behind almost all existing local fishing regulations. Specific rules have been designed to protect coral reef habitats in many sites of social and cultural importance, which have in turn mitigated fishing pressure on certain resources.

This case study suggests that cultural considerations can contribute to the design of management projects: they may be the main objectives of local fishing rules and the primary incentive for participative initiatives and modern management of fisheries. Revitalizing TEK would thus be central to an efficient culture-based strategy for fishery management. The strengthening of TEK and its social and cultural underpinnings may help to lever up ecological awareness because of its significance for local people and their customary values (Berkes 2008). This would indirectly increase the non-use and conservation value of the marine ecosystem of Ouvéa and help to balance economic and conservation interests in the future. Following various authors, our observations confirm that resource management in Oceania should rely on local socioeconomic conditions (Cinner 2007; Cinner et al. 2005) and customary marine tenure systems (Aswani 2005) and social structures (Horowitz 2008; Leblic 2008).

There may also be an important role for TEK and traditional values to play in fisheries management at scales beyond the community level on Ouvéa (Berkes 2006). Whereas, in the past, passive conservation of Ouvéa marine resources was ensured by the low human population density, limited commercialization of marine resources, and simple fishing technology, these are now changing. As a result fishers are now increasingly targeting the lagoon area that had not been traditionally managed. Such activities have not been controlled by public authorities because of a lack of enforcement of official regulations. Thus up-scaled measures are now required to regulate new emerging uses threats to marine resources (e.g. cruise ship tourism, developing commercial small-scale fisheries, and ciguatera poisoning), which could complement the existing small locally-managed areas. Such up-scaling of management and the application of TEK would require a change in governance to involve customary as well as public authorities in a collaborative management framework (Cinner and Aswani 2007). In this context, the addition of Ouvéa to the UNESCO World Heritage List in July 2008 provides a unique opportunity to use TEK as a tool for an innovative management of coral reef fisheries and ecosystems at the island scale. It would also engage traditional leadership in the linked conservation of the island's natural and cultural heritage. In this sense this nomination has formally and internationally recognized the strong linkage between culture and fisheries sustainability in Melanesia.

Acknowledgement

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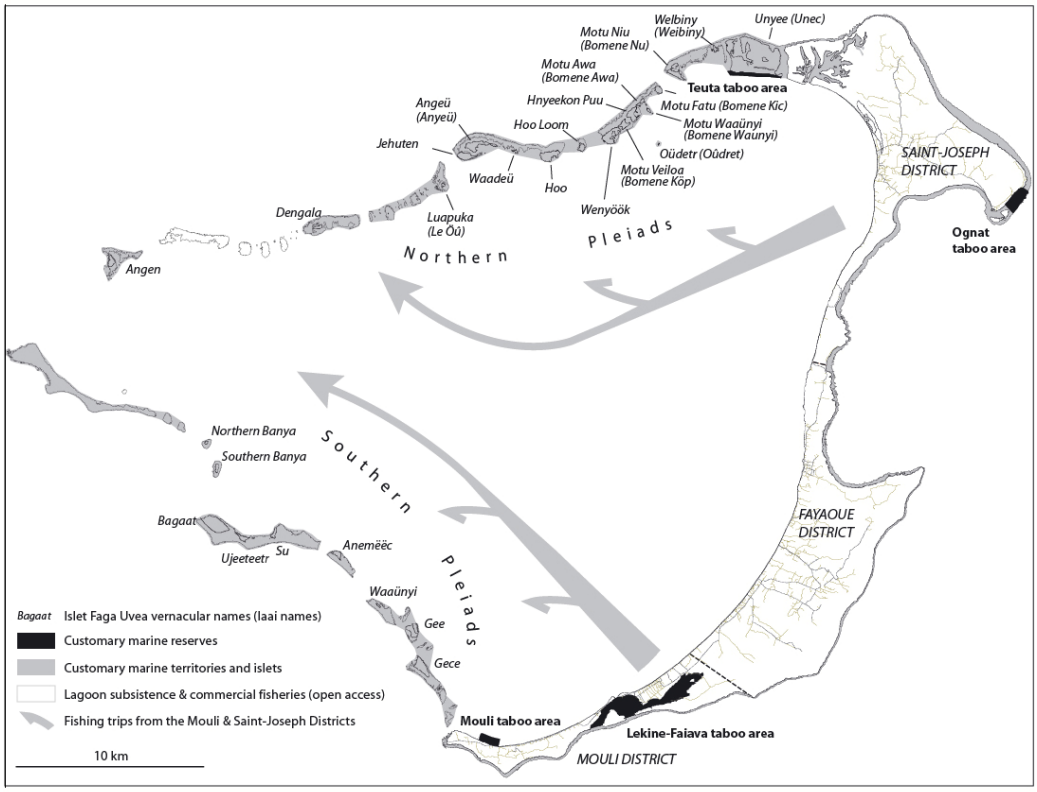

Figure 3. Spatial distribution of community-managed and open access fisheries of Mouli and Saint-Joseph Districts in Ouvéa atoll

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Managing fishing gear to encourage ecosystem-based management of coral reefs fisheries

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Abstract. We present fisheries landing data from two tropical countries, Papua New Guinea (PNG) and Kenya, and show that each gear type causes a unique and consistent partitioning of the species and functional groups it targets. Partitioning by gear can be used to influence ecological processes and biodiversity on coral reefs and to respond to disturbances such as coral bleaching. Hook and line capture a higher proportion of top carnivores and piscivores and target species with low susceptibility to coral bleaching. Traps, drag nets, and spearguns capture mostly herbivores and omnivores and target the highest proportion and number of fish species that are moderately susceptible to the effects of coral bleaching. We argue that the use of specific gears can be actively managed to encourage the recovery of select functional groups and adaptively manage for reducing high erect algae cover and sea urchin dominance, increasing coral cover, and reducing detrimental effects of coral bleaching. We present a simulation and conceptual model that describes the effects of gear and effort on fisheries yields and suggest a mechanism for practically implementing this model. Spearguns are predicted to have the highest diversity and yields of catch, but can also result in decline in herbivorous fishes, which has serious consequences for recovery and resilience of the ecosystem. Further understanding of fishing gear effects on specific fish functional groups at a broader selection of reef locations will be valuable in developing adaptive gear-based management in a changing climate.

Key words: Climate change, coral reef model, ecological effects, fisheries yields, herbivory, trophic cascades

Introduction

Coral reef ecosystems are complex and therefore difficult to manage from a holistic systems approach when social conditions do not facilitate the use of large fisheries closures. Suggestions for management based on an understanding of coral reef ecology are a useful first step but their implementation often depends on complex and challenging socioeconomic considerations (McClanahan et al. 2008). Finding appropriate ways to implement management measures that can be adopted by fishing cultures with resultant desired ecological consequences is perhaps one of the greatest challenges for ecosystem-based management of coral reefs. Management of fishing gear can potentially achieve this goal in areas where fisheries closures are impracticable. Gears are selective in their catch and are a management option mostly accepted by fishers and managers (McClanahan et al. 2005).

The impact of climate change and coral mortality on reef ecology, fish, and fisheries are multiple and complex. Hence, there is a need to evaluate fishing and gear-use scenarios with ecological models based on a holistic view of the ecosystem and existing field data. These models can assist researchers in testing and predicting outcomes and encourage an adaptive management program for coral reef ecosystems. Current understanding of coral reef fisheries has focused on the potential effects of the loss of grazers and invertebrate-eating carnivores (McClanahan 2006). Many investigators believe that the protection of coral reef grazers will be important for maintaining the recovery of coral reefs after disturbances (Hughes et al. 2003; Bellwood et al. 2004; Mumby et al. 2006). Additionally, coral-eating invertebrates, such as Crown-of-Thorns starfish and algae

grazing sea urchins can become pests and compromise ecological processes and recovery potential in some reef environments (McClanahan et al. 2002; Dulvy et al. 2004). These aspects of management were previously examined in an ecosystem model assuming choice of functional groups by fishers (McClanahan 1995).

The objective of the present paper is to examine the potential benefits of gear management strategies to maintain selected ecological processes and enhance reef recovery after coral depleting disturbances. We analyze the composition of fish catches in artisanal fisheries in two countries with different gear uses and levels of fishing effort and evaluate the effects of gear–specific fishing intensity on functional groups of reef fishes.

Materials and methods

The study examines fishing gear and catches in two countries that represent a low to moderate level of fishing (Papua New Guinea; PNG), and moderate to high level of fishing, (Kenya; Cinner and McClanahan 2006; McClanahan et al. 2008). We evaluate the species and functional group selectivity of gear used in artisanal fisheries (net, speargun, beach seine, line and trap) in these two countries and use these data to calibrate an ecosystem simulation model. Model simulations are then used to forecast catch and stock status of selected functional groups under increasing fishing pressure for the different gears.

Field studies of catch by gear

Catch data are based on six fishing sites in PNG (Cinner and McClanahan 2006) and ten fish landing sites in Kenya (McClanahan et al. 2008). Landing sites were selected to represent a wide range of social, economic, and demographic conditions. The fisheries were typical tropical artisanal fisheries where catch was derived from shallow-water coral reef and seagrass ecosystems. Analyses of catch data are based on 4205 and 2154 fish specimens from Kenya and PNG, respectively.

Methods to sample fish catch in the two countries differed slightly. In both countries we opportunistically examined fish landings at all times of the day and night by approaching and asking permission from fishers as they returned from fishing activities and the whole catch was measured whether the catch was for market or home use. Abundance and taxonomic composition were recorded to species level (Lieske and Myers 1994; Randall et al. 1997). In PNG, we photographed the fish using the methods of Cinner and McClanahan (2006) and recorded the gear used to capture each fish. When multiple gears were used in a single trip, we separated the catch by gear type. In Kenya, catches were identified, counted, and measured to the nearest centimeter using a fixed marker rule on a flat board. Where possible the entire catch was sampled, alternatively a sub sample was measured, ensuring that each gear used at each site was adequately sampled and each species landed was recorded.

Although a variety of fishing gears and techniques were used throughout PNG, three main gear types were widespread and used in sufficient numbers to be useful for management and comparison; line fishing, gill nets, and spearguns. In Kenya, these three gears were also commonly used along with beach seines and traps. The infrequent use of other fishing methods (weirs, poisons, bombs, and derris root) did not allow for sufficient data to make comparisons.

Catch and gear analyses

We used expert opinion to group species into the following functional groups, based on their diet: piscivore, macro piscivore-invertivore, plankivores, macro and micro invertivores, grazer (micro-turfs), macrograzers (seagrass and large erect algae), scraper/excavator (parrotfish that remove coralline algae and calcium carbonate while grazing), and detritivores. We also classified species particularly important for ecological processes as a "key-species", i.e. batfish and red-lined triggerfish (McClanahan 2000, Bellwood et al. 2006). We used ordination plots generated by correspondence analyses to examine how nation and gear were related to the above functional guilds and species.

Simulation model development

A coral reef computer-based simulation model was developed to specifically run scenarios for fisheries management using the above gear types. This model, which has been given the acronym CAFFEE (Coral-Algae-Fish-Fisheries Ecosystem Energetics), is a system-dynamic model of a coral reef ecosystem based on the transfer of energy implicit in interactions between functional groups. The model is an expansion and improvement on a previous model (McClanahan 1995).

CAFFEE was developed in STELLA to represent a conceptualized reef food-web that integrates 17 functionally distinct groups across four trophic levels, including 6 primary producers, 8 primary consumers, 2 secondary consumers and 1 tertiary

consumer (Fig. 1). Interactions between functional groups were modeled using an energy-based approach that considered metabolism and biological processes (production, consumption, assimilation, respiration, excretion), as well as ecological factors such as resource competition, density-dependent consumption, and group-specific rates of capture by different fisheries. A detritus-cycle including benthic and pelagic DOM and POM was also incorporated. Calibration used parameters obtained from the literature or derived from datasets provided by collaborating scientists (a full list of references is available on request). A fuller presentation of the model's equations and calibration is forthcoming.

Simulations in the absence of fishing effort ('pre-fisheries' conditions) were allowed to run until a steady state was achieved (i.e. until the values of all stock variables had less than 0.1% of annual variation). The standing stock of each functional group at this stage was taken to be the 'pre-fisheries stock' and these values were used as initial or steady-state stock values (30 to 50 years of simulation) for determining the effect of all further fisheries simulations.

Steady-state stocks of functional groups were compared at different levels of fishing effort with 'pre-fisheries stocks' for different fisheries in order to identify gear-based management strategies likely to enhance reef recovery after disturbance. The model's program increases the catch rates of different gear types at 1 fisher per km^2 intervals and the effect on the stocks on key functional groups were taken and plotted. Gear and catch data from the PNG and Kenya landing sites were used to calibrate the fisheries parameters of the model and here we focus on the effects of effort and gear use on total yields and grazer abundance.

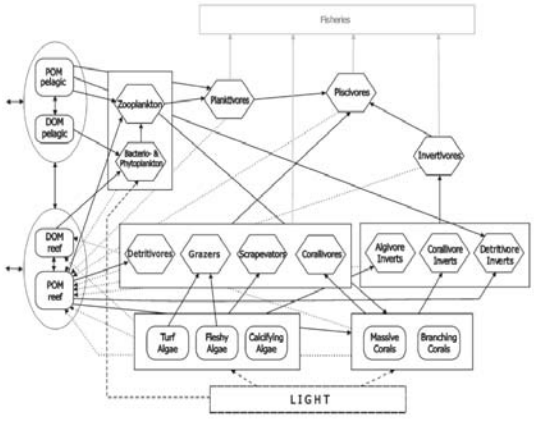

Figure 1. Systems diagram showing the main components and flows of the coral reef ecosystem model used in the gear-use simulation experiments.

Results

Gears were strongly associated with functional groups in both countries (Fig. 2). PNG spearguns caught mostly (micro) grazers, scraper/excavators, detritivores, and planktivores. Kenyan spears, traps, and beach seines caught more grazers (i.e. browsers) and invertivores. Hook and line in both Kenya and PNG captured more carnivores but Kenyan catches had fewer planktivores. Set or gill nets predominantly caught carnivores. Nets in PNG caught piscivores and macro-invertivores, whereas Kenyan nets caught more micro-invertivores and some grazers.

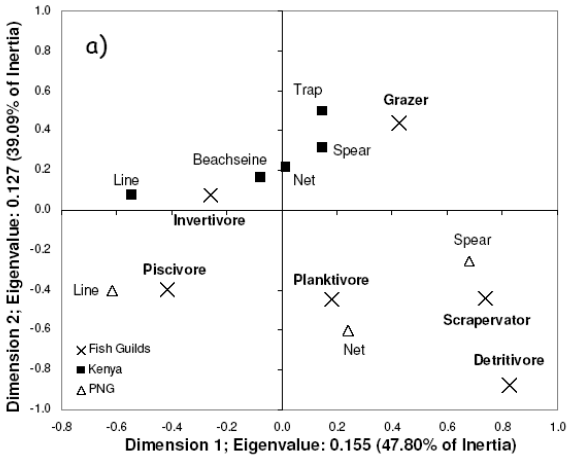

Figure 2. Correspondence analysis plot showing the relationships between functional group catch by gears in the two countries. Scrapervator = scraping and excavating parrotfish.

Model simulations using the calibration for the PNG catch produced three distinct responses for catches by the gear types - spears, nets, and hook-and-line (Fig. 3). Spears produced the highest yield but it was also associated with a collapse in the fisheries beyond the maximum catch, which for this simulation calibration was found at 12 fishers km^{-2}. Nets produced the second highest yields and above 25 fishers km^{-2} did not increase the total yield. Hook and line produced the lowest total yields and declined above 25 km^{-2} for increasing fishing effort, suggesting a constant diminishing catch per fisher. Model outputs calibrated for all gears and pooling all grazers together into a single functional group indicate that spearguns, traps, Kenyan gill nets, and beach seines will reduce grazers to extinction at moderate levels of fishing effort while grazer fish stocks will be able to

sustain greater fishing pressure by lines and the nets used in PNG (Fig. 4).

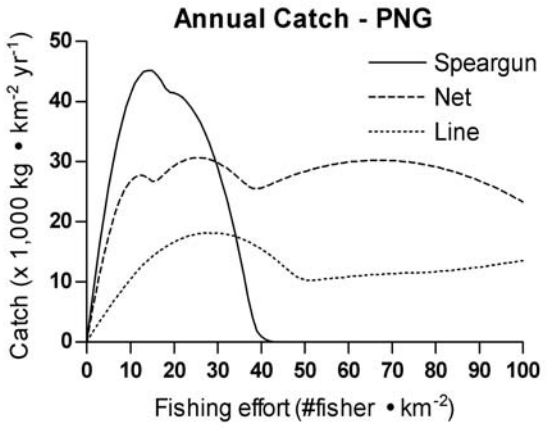

Figure 3. Estimated sustainable annual catch for three gear types from simulation runs based on the Papua New Guinea model calibration.

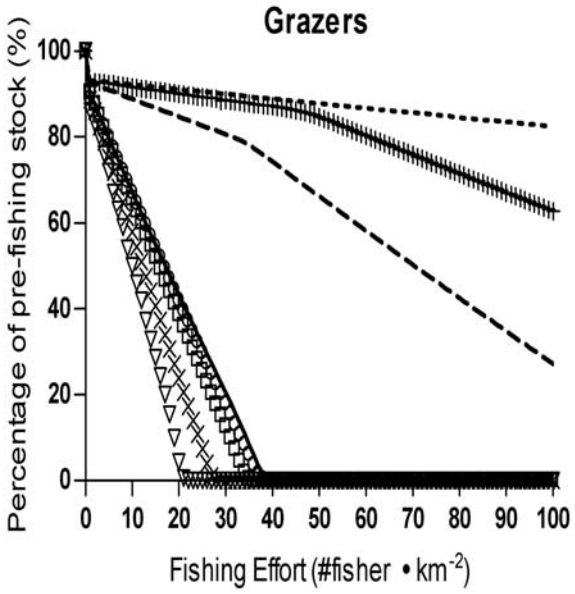

Figure 4. Long-term effects of the common gear types on the grazer functional groups. Legend: (-) line PNG, (+) line Kenya, (—) net PNG, (•) spear PNG, (°) net Kenya, (□) beach seine Kenya, (×) spear Kenya, (∇) trap Kenya.

Discussion

Field data on fish catches by gear in the two countries indicates strong associations between the functional groups of caught fish and types of gear used with some differences in the two nations. We expected the effect of "nation" due to the different levels of fishing intensity and history in the two countries. Kenya has higher numbers of fisher and fishing intensity than PNG. With regard to fisheries yield, the main difference between Kenya and PNG was the high amount of grazers, particularly macro-grazers, caught by spearguns, beach seines, and set nets in Kenya. Many of these macro-grazers feed in and on seagrass and erect algae, and national differences are likely a result of greater use of seagrass ecosystems and greater fishing pressure in Kenya (McClanahan et al. 2008).

Spearguns largely catch herbivores of various kinds in both countries, and a considerably high diversity of other species, as demonstrated by catch-biodiversity studies (McClanahan and Cinner 2008). This high diversity of catch is likely to explain the rapid decline in catch at high levels of effort found in speargun simulation models. The high diversity of catch allows more resources to be utilized but the ultimate consequence of this versatility is a potential for total fisheries collapse at high levels of fishing effort. Fisheries involving gear with low catches of grazers did not collapse but the catch per fisher is extremely low at high levels of effort for PNG nets and hook and line. It is likely that fishers will switch to more effective gear as yields drop, and this may lead to greater use of beach seines and spearguns, with associated losses of grazers (Pauly et al. 1989; McClanahan et al. 2008). Catching fish higher in the food web may not result in the loss of grazers and may help conserve corals, but it is also associated with lower yields. These yields are, however, more stable and less likely to collapse. Lower, yet sustainable, yields are indicative of the types of trade offs required in managing fisheries.

It would appear that gears effective at catching reef grazers should be avoided in order to prevent their population collapse and possible degradation of reefs associated with low grazing. This has not occurred in Kenyan reefs because grazing sea urchins have largely occupied the niche of grazing fish (McClanahan 2008). The catch of reef grazers has also been stable in Kenya, as most catch is now derived from seagrass ecosystems that appear less likely (or possibly slower) to collapse or to become dominated by sea urchins (Heck and Valentine 2007; McClanahan et al. 2008). Where overfishing effects are not buffered by seagrass and sea urchins, we may expect to see degradation of coral reef ecosystems and collapses of fish populations at lower levels of effort, especially where the most effective or destructive gear are not controlled. We

suggest that knowledge of fishing gear effects can be used to develop a conceptual basis for adaptive ecosystem-based coral reef management. Knowledge of functional groups and their effects on reef processes can be utilized to develop a feedback between the state of the reef ecosystem and choices of gear use (McClanahan and Cinner 2008).

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Indications of Recruitment Enhancement in the Sea Urchin *Tripneustes gratilla* Due to Stock Restoration Efforts

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Abstract Field monitoring activities were conducted in various sites in five provinces in northwestern Philippines in 2004-2007 to assess the impacts of restocking and grow-out culture of hatchery-reared *Tripneustes gratilla* juveniles. High incidences of recruits (<4.0 cm test diameter) were noted during the last quarter of 2005 through the first quarter of 2006. Very high densities (up to 460 individuals 100 m^{-2}) were recorded in Lucero, Bolinao, Pangasinan, which is the site for sea urchin broodstock and grow-out cages. Eighty percent of the total density was comprised of recruits of the year. In Sinait, Ilocos Sur, total density and incidence of recruits were significantly higher (up to 67 individuals 100 m^{-2}) than two sites in Ilocos Norte where there was no grow-out culture of sea urchins. In Poro Pt., La Union sea urchins increased after the initiation of grow-out culture activities in 2005 and peaked at 14 individuals 100 m^{-2} in 2007. The positive correlation in the density of recruits and adults in four sites in Bolinao together with the higher densities and incidence of recruits in different grow-out areas concur with the hypothesis that the aggregation of adults in grow-out culture areas enhanced benthic recruitment.

Keywords: recruitment, sea urchins, grow-out culture, stock restoration

Introduction

The reef flats of the coastal municipality of Bolinao, Pangasinan were a prime sea urchin fishing area for *Tripneustes gratilla* in the 1970s through the late 1980s. Unregulated harvesting precipitated in the collapse of the fishery in 1992 (Juinio-Meñez et al. 1998). Hatchery culture was initiated in 1994 and juvenile production was scaled-up by 1996 for restocking to enhance the recovery of the sea urchin fishery in Bolinao. To optimize survivorship of the limited numbers of hatchery-reared juveniles to adulthood, community-based grow-out culture was developed as an adaptive resource management tool to address both ecological and socio-economic considerations while rebuilding the spawning population (Juinio-Meñez et al. 1998). From 2000 to 2006, about 50,000 juveniles were used annually for restocking in selected protected areas and grow-out culture in the Bolinao area. After the Bolinao fishery collapsed in 1992, no natural recruitment was evident until 1999 when a few juveniles were first reported again. During the last quarter of 2004, a strong recruitment pulse was noted together with the increase in the landed catch of collectors, indicating recovery of the natural population in Bolinao. This was further boosted by an even stronger recruitment during the last quarter of 2005 through the first half of 2006 (Juinio-Meñez et al. 2008).

Sea urchin grow-out culture in sea cages was promoted and expanded in the provinces of Ilocos and La Union using wild juveniles to reestablish viable spawning populations and provide a supplemental source of livelihood for fishers in collaboration with fisher's organizations, local government units, regional state universities and the Bureau of Fisheries and Aquatic Resources (BFAR). Among these sites were Sinait, Ilocos Sur and Poro Point, La Union. In Sinait, Ilocos Sur, three to six cages with 500-700 sea urchins per cage were maintained near a marine protected area since 2004. In Poro Point, La Union, a total of 30,000 juveniles (i.e. 60 cages stocked at 500 urchins per cage) were used for the grow-out cages starting December 2005.

Methodology

Data on densities and size frequency distributions of *T. gratilla* derived from field monitoring activities in 2004 -2007 in selected sites in NW Luzon (Fig. 1) were analyzed to gain more insights on the ecological impacts of restocking and grow-out culture of sea urchins.

The total density of sea urchins (individuals 100 m^{-2}) and the density of recruits (<40 mm TD, individuals 100 m^{-2}) at four sites in Bolinao, Pangasinan (Lucero, Silaki Is., Victory and Balingasay, see map inset in Fig. 2) in

2004 to 2007 were compared. Three belt-transects (100 m x 2 m) covering an area of 600 m^{-2}, were surveyed. Sea urchins found within the belt transects were counted and test diameter (TD) was measured to the nearest millimeter using a Vernier caliper. To determine broad scale patterns in recruitment, 6 other locations were surveyed along NW Luzon in January to February 2006 coinciding with the incidence of very high densities of recruits in Bolinao. The four localities surveyed north of Bolinao were Santiago and Nalvo in Ilocos Sur, Paraoir and Poro Point in La Union (Fig. 1). There was on-going grow-out culture of sea urchins in these sites. Whenever possible, additional transects were laid at different distances (10 m apart up to 50 m) from the area of the grow-out cages and another transect in an area with no grow-out cages. South of Bolinao, surveys were conducted at the Hundred Island National Park in Alaminos, Pangasinan (inside Lingayen Gulf) and at three sites in Masinloc, Zambales (Fig. 1).

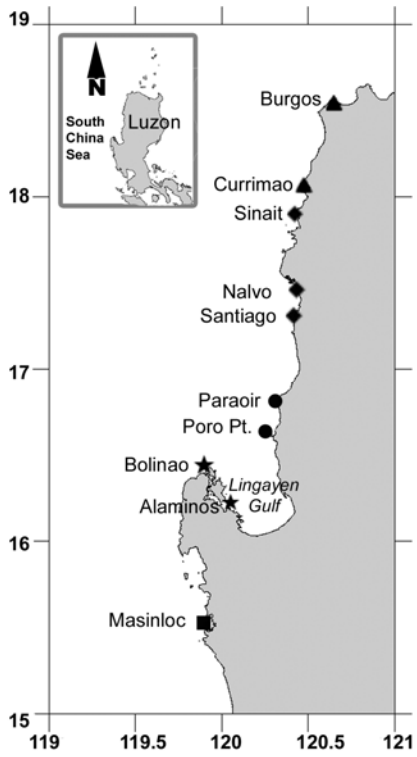

Figure 1. *T. gratilla* monitoring sites in Northwestern Luzon, Philippines. Site legends indicate localities in five provinces: triangle – Ilocos Norte, diamond – Ilocos Sur, circle – La Union, star – Pangasinan, square – Zambales.

Additional surveys were conducted in Poro Pt. in 2006 and 2007 to validate the reported increase of recruits in the area after the initiation of sea urchin grow-out culture. These were compared with densities from a previous survey in December 2003. Monitoring surveys were conducted in three other localities in the Ilocos region (i.e. Sinait, Currimao, Burgos, Fig. 1) from November 2006 to August 2007 in 3 permanently marked sites (2 m x 20 to 50 m), to compare densities and size structure of *T. gratilla* populations.

Results

Variation in Recruitment Strength Among Sites

In Bolinao, >10 individuals 100 m^{-2} was observed for the first time in 2004 since the collapse of the fishery in 1992. The highest density recorded was 460 individuals 100 m^{-2} (of which 80 % were recruits) in Lucero in January 2006 (Juinio-Meñez et al., 2008). Overall, total densities and density of recruits in the three other sites were significantly lower ($p<0.05$) than those in Lucero for the same sampling periods in 2004 to 2007. Notably, there was a positive relationship between the average density of recruits and adults at the four sites during surveys in 2004-2007 (Fig. 2).

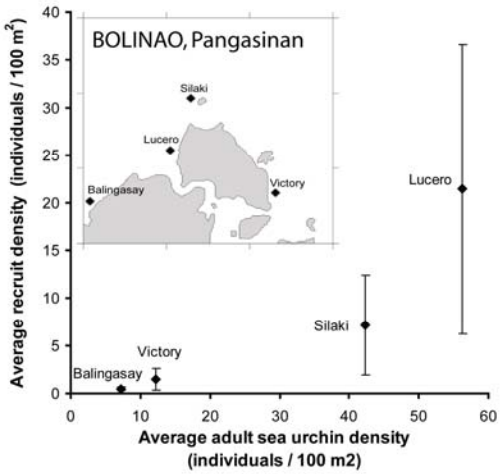

Figure 2. Average density of *T. gratilla* recruits plotted against the adult average densities in Bolinao sites.

A survey in other locations along NW Luzon in early February 2006 showed the presence of recruits in all sites except at the Hundred Island National Park in Alaminos. The modal size class of all sea urchin populations ranged from 3-5 cm TD (Fig. 3). This suggests that the recruits of the year were primarily from larvae, which settled during the previous 3-5 months (September–November 2005), coinciding with the peak of the SW monsoon.

Total density and density of recruits in the sites surveyed in Ilocos Sur, La Union and Zambales during the same period ranged from 10-60 individuals 100 m^{-2}, about an order of magnitude lower than that in Lucero, Bolinao. The harvesting of juveniles for grow-out culture in these areas confounded the estimated densities in Ilocos and La Union. Nonetheless, the density of sea

urchins decreased with distance from the grow-out cages in Paraoir and Santiago (data not shown). This observation was consistent with anecdotal accounts of fishers engaged in grow-out culture. In Poro Point and Nalvo, growers also observed higher numbers of juvenile sea urchins near the cages. However no pattern was evident from the field survey results.

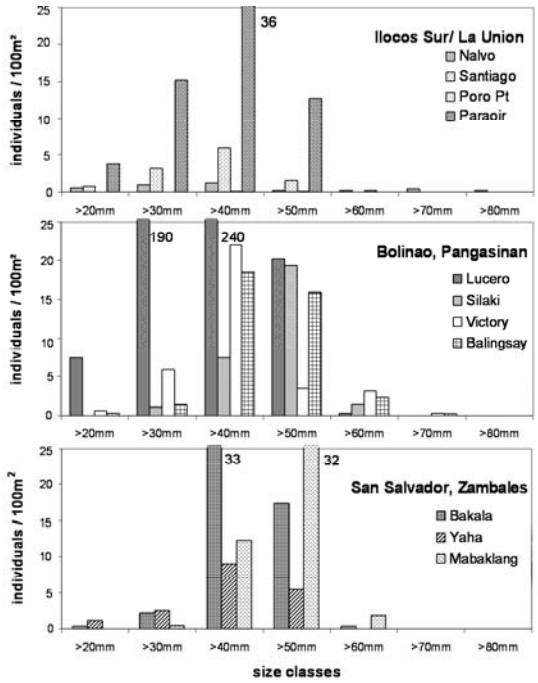

Figure 3. Size frequency distribution of sea urchins in various sites in Northwestern Luzon on January - February 2006.

Monitoring surveys in other sites in the Ilocos region showed significant differences in mean density of *T. gratilla* among the three sites surveyed. Mean densities in Sinait were significantly higher compared to Currimao and Burgos (Table 1). During the November 2006 and February 2007 surveys, early juveniles (10-30 mm TD) comprised about 67% of the total sea urchins sampled in Sinait. Overall, juveniles (< 40 mm TD) were more abundant in Sinait during most sampling months while in Currimao and Burgos, late adults (<70 - >90 mm TD) urchins were more abundant.

Recruitment before and after initiation of grow-out culture in sea cages

Densities of *T. gratilla* increased in Poro Pt., after the initiation of grow-out culture in December 2005 (Fig. 4). Starting September 2006, juveniles comprised 79-83% of the total counts, validating the report of locals on the high incidence of juveniles. Prior to grow-out culture, density of sea urchins in the area was very low. In fact, juveniles for the sea urchin grow-out cages were sourced from a neighboring town (Paraoir). Higher densities of juveniles were found in the monitoring sites inside the embayment. However, there was no positive relationship between abundance of juveniles relative to distance from the grow-out cages up to 50 m.

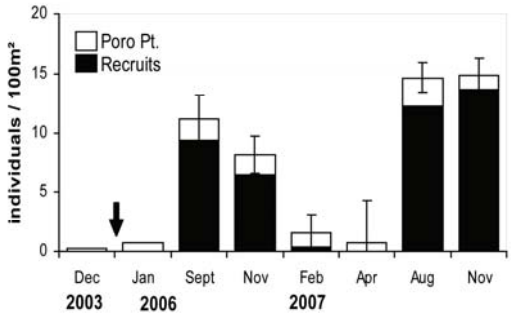

Figure 4. Mean sea urchin density in grow-out site in Poro Pt., La Union. Arrow indicates the start of grow-out culture.

Discussion

The positive correlation in the density of recruits and adults in the four sites in Bolinao, the high incidence of recruits in Lucero and Sinait relative to other sites without sea urchin grow-out culture, and the significant increase in the density of juveniles after the initiation of grow-out culture in Poro Pt. strongly indicate that the aggregation of adults in the grow-out cages enhanced benthic recruitment. In addition, the higher density of sea urchins near the grow-out cages in Paraoir and Santiago during the February 2006 survey validated the anecdotal accounts of fishers engaged in grow-out culture.

Similarly, recruitment strength of *Diadema antillarum* in Barbados was positively correlated with adult densities. Juvenile mortality was low in adult settlement sites and juveniles aggregated in reefs with adult populations. These suggested that regionally dispersed larvae may prefer areas of high adult density (Hunte and Younglao 1988). Compounds from conspecific sea urchins can act as settlement cues for larvae (reviewed in Hugget et al. 2006; Swanson et al. 2006) as described in several benthic marine invertebrates (reviewed in Rodriguez et al. 1993).

Table 1. Overall mean density of *T. gratilla* (individuals 100 m^{-2}) in the three study sites in Ilocos over a ten-month sampling period. Means with the same letters are not significantly different from each other (ANOVA p>0.05, Tukeys HSD test). Standard error values are in the parentheses.

	Nov. '06	Dec. '06	Jan. '07	Feb. '07	May '07	Aug '07	**Mean** (s.e.)
Currimao	17.29	13.97	13.05	19.25	0	80.98	**24.1** [b] (11.7)
Sinait	29.37	49.06	47.08	184.79	70.52	22.18	**67.2** [a] (24.5)
Burgos	7.00	12.00	7.00	16.67	8.33	5.67	**9.4** [c] (1.7)
Mean (s.e.)	**17.88** (6.46)	**25.01** (12.04)	**22.37** (12.47)	**73.57** (55.61)	**26.28** (22.25)	**36.28** (22.85)	

Dworjanyn and Pirozzi (2008) found that *T. gratilla* larvae settled at a higher rate in the presence of live juveniles and their faeces. Settlement near conspecifics provides the advantage of settling in a habitat that can more likely support post-larval growth (reviewed in Pawlik, 1992) and for some species, may provide protection from predation as observed for *Strongylocentritus franciscanus* wherein juveniles were protected by adult spine canopies (e.g. Tegner and Dayton 1977; Tegner and Dayton 1981).

In general, gregarious behavior and high densities in echinoids have been reported to increase fertilization success (Pennington 1985; Levitan 1991; Levitan et al. 1992; Levitan 2004). Likewise, a population genetic study on the red sea urchin *Strongylocentrotus franciscanus* suggests that high population densities are required for successful fertilization (Burton 2003). Clearly, behavior and critical thresholds in density are important considerations in the establishment of marine protected areas (e.g. Dayton et al. 2000). The very high recruitment success of *T. gratilla* in Lucero compared to other sites in Bolinao appears to be due to the synergy of favorable fin scale biophysical factors and local management interventions that enhanced build-up of the spawning population. In particular, the maintenance of the broodstock for the hatchery and juvenile grow-out cages, the strict enforcement of the marine sanctuary and prohibition of harvesting of small-sized sea urchins in the area reestablished adult populations (Juinio-Meñez et al. 2008).

Since the sea urchins in the grow-out cages are not harvested until they are sexually mature (>6 mm TD) and aggregations in the cages may enhance fertilization success, they maybe local sources of larval supply. The marked increase of recruits in Poro Pt. after the initiation of grow-out culture may be attributed to the high entrainment potential in this site. Based on coastal rugosity and wake formation studies, both Poro Pt. and Bolinao were identified to have high entrainment potential that could concentrate larvae locally and facilitate self-recruitment (Magno 2005). Swearer et al. (2002) proposed that positive stock-recruitment relationships over small spatial scales (e.g. 10s kms), which have been documented for species with a wide range of pelagic larval development periods, are indicative of self-recruitment. Moreover, stronger evidence for self-recruitment based on positive stock-recruitment relationships may be derived from the quantification of local recruitment following localized stock enhancement, as evident in the results for Bolinao and Poro Pt. in this study.

On a broader scale, the modal size range for the different populations monitored during January and February 2006 indicate similar temporal patterns in reproduction of populations in the region. The recruits (3-5 mm TD) are likely from spawning events in May-August based on juvenile growth rates and larval duration under culture conditions (Juinio-Meñez and Hapitan 1998). Larval dispersal modeling studies in NW Luzon suggest that *T. gratilla* populations are highly connected by larval transport due to the reversing monsoons (Juinio-Meñez and Villanoy 1995). Population genetic studies using allozyme also provide some evidence of extensive gene flow between *T. gratilla* populations (Malay et al. 2002). Thus, while self-recruitment is highly likely in some sites like Lucero and Poro Point, there is also high larval exchange among populations in NW Luzon.

Given the high potential for larval exchange among localities and the reproductive biology of this species, establishment of a network of marine protected areas and grow-out culture are strategic in conserving larval sinks and sources for *T. gratilla* in the region. Further investigations on fine-scale and meso-scale hydrographic conditions together with genetic studies using DNA markers may further elucidate factors that influence the recruitment dynamics of populations and provide inputs to ensure the sustainability of the fisheries in NW Luzon.

Acknowledgements

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The coupling of St. John, US Virgin Islands marine protected areas based on reef fish habitat affinities and movements across management boundaries

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Abstract
NOAA's Biogeography Branch, National Park Service (NPS), US Geological Survey, and the University of the Virgin Islands (UVI) are using acoustic telemetry to quantify spatial patterns and habitat affinities of reef fishes. The objective of the study is to define the movements of reef fishes among habitats within and between the Virgin Islands Coral Reef National Monument (VICRNM), the Virgin Islands National Park (VIIS), and Territorial waters. In order to better understand species' habitat utilization patterns among management regimes, we deployed an array of hydroacoustic receivers and acoustically tagged reef fishes. A total of 150 fishes, representing 18 species and 10 families were acoustically tagged along the south shore of St. John. Thirty six receivers were deployed in shallow nearshore bays and across the shelf to depths of approximately 30m. Example results include the movement of lane snappers and blue striped grunts that demonstrated diel movement from reef habitats during daytime hours to offshore seagrass beds at night. The array comprised of both nearshore and cross shelf location of receivers provides information on fine to broad scale fish movement patterns across habitats and among management units to examine the strength of ecological connectivity between management areas and habitats.

Key words: Acoustic Tracking, Coral Reef, Fish, Habitat Utilization, Telemetry

Introduction

Study Area and Background

Coral reefs in the US Virgin Islands and in Virgin Island National Parks have declined in recent decades. The establishment of the Virgin Islands Coral Reef National Monument (VICRNM) in 2001 provides approximately 5,143 hectares of additional NPS marine managed area off the island of St. John, USVI. To assess the long-term effectiveness of management regulations and VIIS and VICRNM as marine protected areas (MPAs) it is necessary to conduct investigations that can provide data on the movement of reef fishes within areas across and outside NPS boundaries. NOAA digital benthic habitat maps coupled with movement patterns of fishes provide a spatial framework to address questions concerning linkages among adjacent habitats and how the mosaic of habitats connect in the seascape that structure reef fish distribution patterns.

Material and Methods

Island Scale

To determine the movements of reef fishes along the entire south shore of St. John and portions of its eastern bays within and outside VICRNM and VIIS, 36 receivers were deployed (Fig. 1).

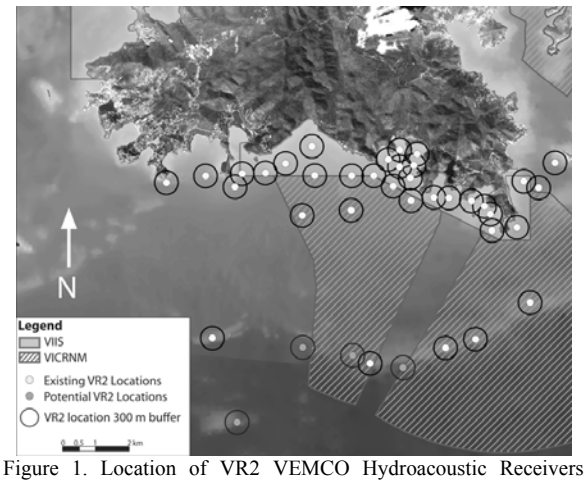

Figure 1. Location of VR2 VEMCO Hydroacoustic Receivers around St. John, USVI.

Each of the acoustic receivers has a nominal detection range of 300m. Based on our knowledge of the distribution of habitats and reef fish ecology, we

deployed receivers in shallow nearshore bays and across the shelf to depths of approximately 30m. Receivers were located within reefs and adjacent to reefs in seagrass, algal beds, or sand habitats and within and outside of VIIS and VICRNM. This array provides data to define "island scale" patterns of reef fish movements.

Fine Scale

To define fine scale movement of reef fishes and their habitat affinities, of the 36 receivers found within the entire array, 9 of these receivers were concentrated in the Lameshur Bay complex. The receivers were deployed to ensure "overlap" among detections of acoustically tagged fishes and to ensure all habitats within the bay were covered by the fine scale array (Fig. 2).

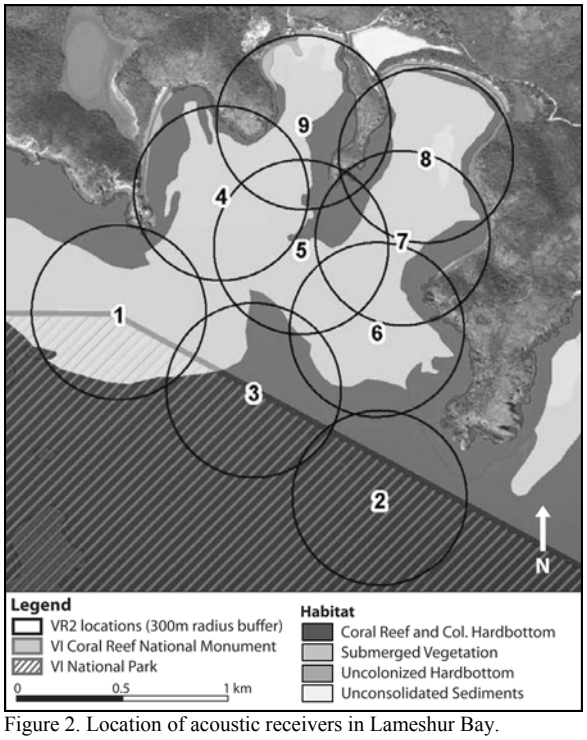

Figure 2. Location of acoustic receivers in Lameshur Bay.

Acoustic Tagging

We surgically implanted VEMCO V9-2L-R64K transmitters into the stomach cavities of captured fishes (Fig. 3). A 1cm incision was made 1cm off-center from the ventral midline behind the pelvic fins and a small acoustic transmitter (22mm) was placed within the body cavity. The incision was closed with two surgical sutures and the fish were observed to ensure adequate recovery. After holding fish for 24 hours in post surgery recovery tanks, they were released at a location in close proximity to their original capture location.

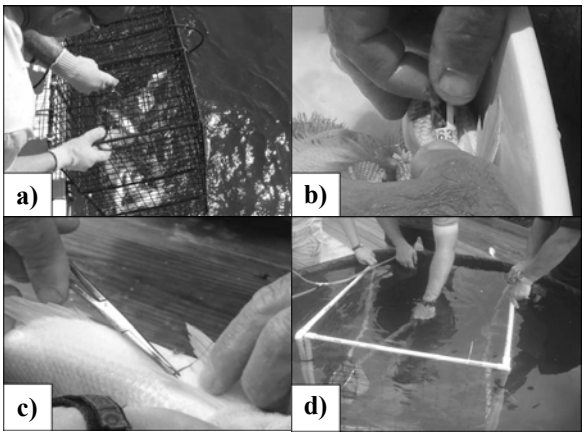

Figure 3. a) Fish traps used to capture fish; b) surgical implanting of acoustic transmitters; c) closing incision with surgical suture; and d) a crowder used to minimize handling and stress in the holding/ recovery tank.

Results

Lameshur Bay-wide Habitat Utilization

The greatest number of total detections were recorded at Station 5 (55%), followed by Station 3 (20%), and Station 6 (16%), respectively (Fig. 4). This is despite the fact that 51% of all of the releases during this period of the study (July 2006 to April 2007) occurred at Station 6, with 27% released at Station 5 and 22% at Station 4. In addition, the receiver at Station 5 stopped collecting data after December 15, 2006 due to memory limitations. The dense seagrass bed adjacent to Yawzi Point (Station 5) may have resulted in shorter migration distances to nighttime foraging areas that were still within the detection range of Station 5 (Fig. 5).

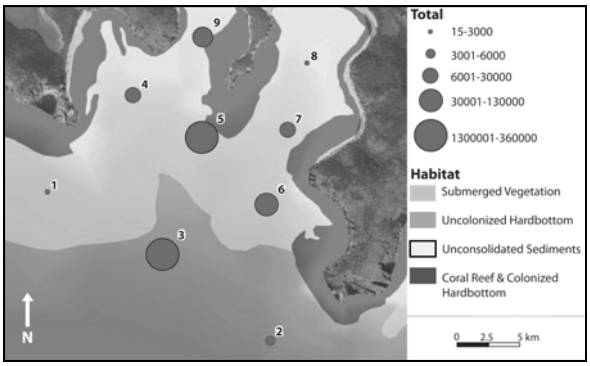

Figure 4. Total number of detections by receivers from July 2006 to April 2007.

*Individual Fish Movement – Lane snapper (*Lutjanus synagris*)*

Figure 6 is a plot of receiver detections for an individual lane snapper (29cm TL) undergoing sun-synchronous migrations into and out of Lameshur Bay between July 12, 2006 and April 5, 2007 (268 days; night= 0:00-4:00 and 20:00-24:00, crepuscular= 4:00-8:00 and 6:00-20:00, daytime=8:00-16:00). The

data indicate daytime site fidelity with the eastern side of Lameshur Bay and a regular departure from the bay at sunset and a return to the bay at sunrise.

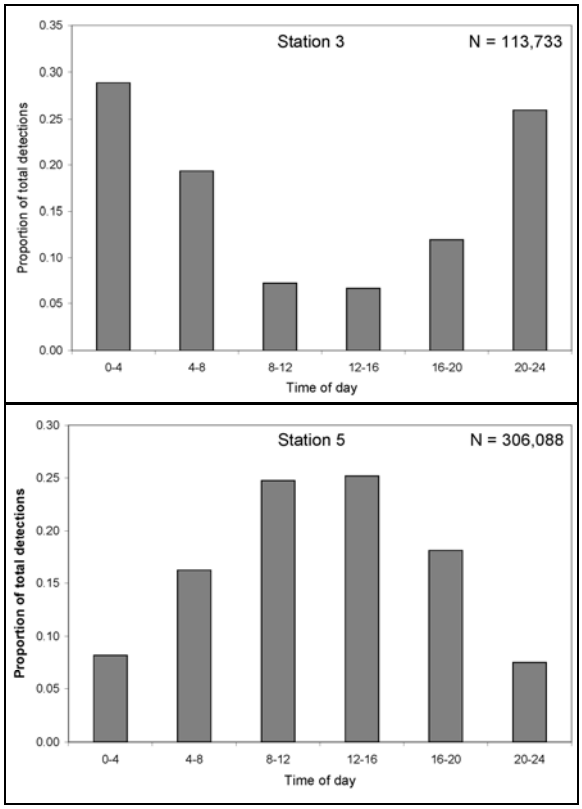

Figure 5. Proportion of total detections by time of day for: a) Station 3 and b) Station 5.

This movement pattern is further highlighted in Figure 7 that shows at approximately sunset this fish was detected at Station 2 and was likely leaving the bay since it was not detected on any other receiver at night. It appears the fish migrated offshore into deeper water to presumably to forage and returned back to Lameshur Bay at sunrise.

Discussion

The joint NOAA, NPS, USGS, and UVI acoustic tracking of reef fishes found around St John, USVI is providing a wealth of information to define reef fish movements and habitat utilization patterns. These data have been and will continue to be used to define the ecological connections between the VIIS, VICRNM, and USVI Territorial waters. This work directly supports NPS and Territorial management of living marine resources by evaluating the efficacy of marine protected areas, defining species habitat affinities and determining the temporal patterns of reef fishes at island-wide and fine scale spatial extents.

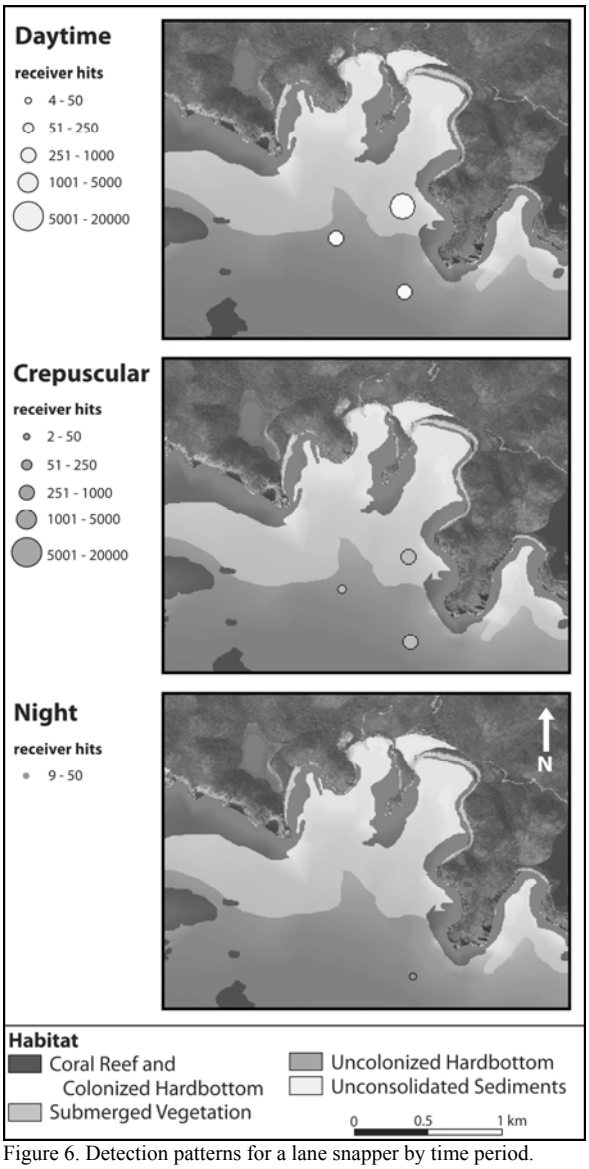

Figure 6. Detection patterns for a lane snapper by time period.

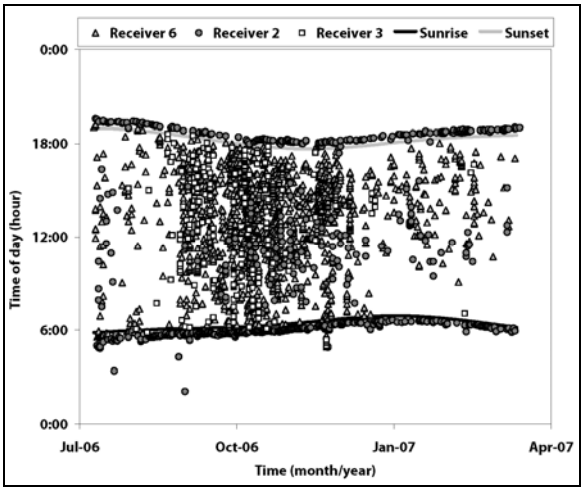

Figure 7. Data for lane snapper indicating sun-synchronous nocturnal migrations and high daytime site fidelity.

The next steps in the work are to continue to analyze the acoustic returns of individual fishes detected across the receiver array and to deploy 4 additional receivers to better elucidate the connectivity between the relatively deep mid-shelf reef area found offshore of southern St. John and within the VICRNM to VIIS and nearshore territorial waters. Plans are to continue the study to at least December of 2009 and then determine if and when to move the receiver array to additional locations within the USVI.

Acknowledgements

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Banggai cardinalfish: towards a sustainable ornamental fishery

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Abstract. Central Sulawesi Province has over 4,500km of coastline and over 700 islands including the Banggai Archipelago. Established as Banggai Kepulauan District in 1999 and covering almost all the native distribution of the Banggai cardinalfish, *Pterapogon kauderni,* though introduced populations have become established elsewhere. A paternal mouth brooder with direct development traded in large numbers as an ornamental fish, *P. kauderni* was recently proposed for listing in CITES Appendix II and has since been listed as Endangered on the IUCN (International Union for the Conservation of Nature) Red List. Local activities to develop sustainable management of *P. kauderni*, underway since 2005, aim to address concerns regarding the Banggai cardinalfish fishery and trade as well as habitat conservation. The CITES proposal in 2007 brought the Banggai cardinalfish into the limelight at national and international levels, and a multi-stakeholder multi-year national Banggai Cardinalfish Action Plan has been developed. Developments since 2004 are outlined including initiatives under the Sea Partnership Program; local research and some recent results; and early progress in implementing the Action Plan, including the establishment of the BCF Centre, District and village marine protected areas and a Banggai cardinalfish trade monitoring system.

Key words: Banggai cardinalfish, Sulawesi, ornamental fishery.

Introduction

The Banggai cardinalfish *Pterapogon kauderni* (Koumans, 1933) is an endemic, reef-associated fish with a distribution limited to the Banggai Archipelago in Central Sulawesi, Indonesia and a few nearby islands (Fig. 1). Some introduced populations have become established along the trade routes, including sites in North Sulawesi (Erdmann & Vagelli, 2001), Palu Bay (Moore & Ndobe, 2007a) and North Bali (Lilley, pers.com).

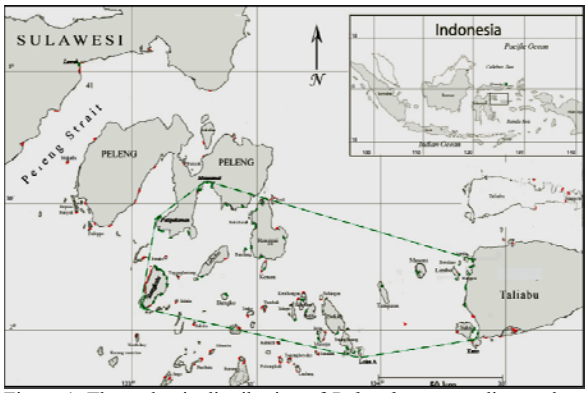

Figure 1: The endemic distribution of *P. kauderni* according to the CITES proposal (Vagelli, 2005 *in* Anonymous, 2007a).

The male incubates the eggs (around 20 days) and larvae (around 9 days) in his mouth, and fasts for this period On release juveniles immediately seek shelter and food in available nearby habitat, with no pelagic dispersal phase, cannibalism by the (male) parent and other adult fish is common (Vagelli, 1999).

Locally known as *bebese tayung*, which in Bajo (sea gypsy) language means little fish in the sea urchins, since the early 1990's the Banggai cardinalfish has been internationally traded as an ornamental fish (Ndobe & Moore, 2007).

Discovered by Kaudern and classified by Koumans in 1933, *P. kauderni* was "forgotten" by the scientific community until it was "rediscovered" in 1994 (Allen & McKenna 2001). In the following 10 years there was much international interest and a considerable amount of research into the biology, ecology and exploitation of the species by visiting scientists who generally considered the trade to be unsustainable (Allen and McKenna 2001; Lunn and Moreau 2004; Kolm and Berglund 2003; Vagelli and Erdmann 2002).

Key data and information were available at international level since 2001 or even earlier but in general were not communicated to local or national

stakeholders and therefore had no impact on management (Ndobe & Moore, 2007).

Material and Methods

The paper presents a synopsis based on literature and on personal experience during the process of local awareness building regarding the Banggai cardinalfish followed by early steps in developing management activities over the period from October 2004 to the 11th ICRS in July 2008.

2004-2005: Initial Awareness Building

A case study in the Banggai Islands was undertaken by the NGO Yayasan Palu Hijau as one of several short case studies in three countries commissioned by NACA (Network of Aquaculture Centres in Asia), coordinated in Indonesia by the STREAM (Support to Regional Aquatic Resources) Hub at the Directorate General for Aquaculture, under the ECPREP (European Community Poverty Reduction Effectiveness Program) project entitled "The International Seafood Trade: Supporting Sustainable Livelihoods Among Poor Aquatic Resource Users in Asia" (EC-PREP, 2005). The main overall project aim was to identify mechanisms for reducing poverty associated with the ornamental fish trade.

The Banggai cardinalfish was found to be the main ornamental species caught by local rather than "outside" fishers and heavily (over) exploited. The fishery was poorly managed from capture through post-harvest handling and husbandry to packing and transport. The market chain was long and complex and trading patterns were often unfair and inefficient. Direct and indirect threats to *P. kauderni* populations and habitat including threats to and from other fisheries were identified. However, observations and data also indicated the possibility of a sustainable fishery. Indeed the highest population density was recorded at a site where collection had been carried out for some years on a periodic basis, with rotation between sites.

The results were presented to stakeholders at District (local), Provincial and National level. The local stakeholders recognised that action was needed. An outline program was agreed to and proposals drawn up.

2006: The First Steps

Implementation of the plans developed in 2005 began in 2006 with support from the Sea Partnership (Mitra Bahari) Program of the Department for Marine Affaires and Fisheries through the Central Sulawesi Regional Centre Sea Partnership Consortium (KMB). The KMB partners include local Universities/Higher Education Institutes and NGOs, as well as local government agencies.

Activities undertaken included a survey of *P. kauderni* populations, habitat and exploitation in Banggai Island, which along with the data from 2004/2005 formed the basis for further local awareness building, in particular through a Marine Protected Area (MPA) Management training and workshop. As a result, two villages (Bone Baru and Tinakin Laut) decided to establish community MPAs and a local organisation for MPA development was founded.

Research into *in-situ* breeding of the Banggai cardinalfish was carried out, based on a model developed by Dr Kolm, involving undergraduate students and the introduced population in Palu Bay (Ndobe & Moore, 2007; Moore & Ndobe, 2007a). The results raised a number of questions and challenged some conclusions in the scientific literature on *P. kauderni*. Further research was planned and is still ongoing.

The key results of these and other activities were included in two books published in Indonesian as part of the KMB 2006 program. One book was on the biology, ecology and conservation of the Banggai cardinalfish and the other on the fishery and trade.

2007: CITES CoP 14 and local impacts

In early 2007 the Department of Marine Affaires and Fisheries contacted the Central Sulawesi Provincial Fisheries Service to request information about *P. kauderni* in connection with the proposal submitted by the United States of America to list the species on CITES Appendix II at the 14th Conference of the Parties (CoP) in June 2007. News of this proposal came as a great surprise to stakeholders in the endemic distribution area who had no idea that such a proposal was being prepared much less already submitted (Moore & Ndobe, 2007b).

The FAO expert panel (FAO, 2007) did not support the listing on scientific grounds. It is now history that the proposal was withdrawn and that Indonesia made a strong public commitment to Banggai cardinalfish conservation (Anonymous, 2007b).

Local Activities pre and post CITES COP 14

In addition to providing data and information to many parties including the Indonesian authorities and delegates, the FAO expert panel, the IUCN working group and others, local activities included research funded by the Higher Education Department (DIKTI) junior lecturer research programme into the phenomena of ontogenetic shift in micro-habitat first put forward by Vagelli (2004).

This research, some of which has since been presented in local and national meetings (e.g. in Ndobe & Moore, 2007), was carried out in the native habitat (three habitat types: coral reefs, seagrass beds and reef flats) around Tinakin Laut, Banggai Island and at the introduced population site in Palu Bay (homogenous habitat). The results revealed no significant difference between habitat types, but

indicated a strong preference of new recruits and small juveniles for sea anemone micro-habitat, even when *Diadema* sea urchins were abundant (Fig.2).

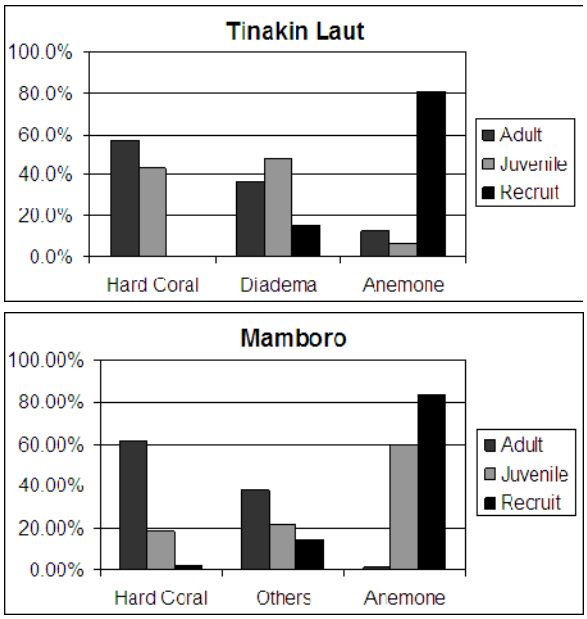

Figure 2: Size/age class distribution by micro-habitat at Tinakin Laut (Banggai Island, top) and Mamboro (Palu Bay, centre))

In addition, the highest numbers of uniform size (apparent sibling) groups of recruits and small juveniles were recorded in anemone micro-habitat. An important management implication is the need to conserve sea anemones, which were overexploited for human consumption.

Hard corals seem important for adults, and (as in 2004 and 2006), some survey locations were under severe attack from a population explosion of the corallivorous starfish *Acanthaster plancii*. The results also raised questions regarding the periodicity of Banggai cardinalfish breeding and indicated that seasonal factors may influence breeding patterns, success or possibly both.

Banggai Cardinalfish Action Plan

After CITES CoP 14, a national level meeting was held in Palu, capital of Central Sulawesi, attended by senior officials from the Department of Marine Affaires and Fisheries, Provincial and District Governments, fishermen, local leaders, local and national NGOs and Academia. A multi-year action plan for sustainable management of the Banggai cardinalfish was drawn up. A synopsis of this plan (translated from the original) is described in Fig 3.

2008: Early achievements and constraints

Initial steps in implementation during 2007 included the establishment of the BCF Centre (Decree No 168/2007 of the Banggai Kepulauan District Head), and a Marine Protected Area (Decree No. 540/2007).

Activities underway and planned for 2008/2009 include:

(i) Support (mainly from the Provincial Fisheries Service in partnership with NGOs and Higher Education Institutes) for the MPAs proposed by the village communities of Bone Baru and Tinakin Laut, in the form of training, physical facilities, etc. It is anticipated that the two community MPAs will become functional during 2009.

(ii) The drafting of a Ministerial Decree regarding the (sustainable) management of the Banggai cardinalfish is in progress, completion anticipated in early 2009

(iii) Development of the BCF Centre, working closely with ornamental fishers and local traders. Despite funding constraints, anticipated activities include socialisation and management planning.

(iv) Ongoing research by local scientists (including the involvement of undergraduate students) into various aspects of Banggai cardinalfish biology, ecology and husbandry, both *in-situ* and *ex-situ,* involving the Banggai Cardinalfish Unit established at the Provincial Fisheries Hatchery Complex at Mamboro in Palu Bay, the Fisheries and Marine Higher Education Institute (STPL-Palu), the Aquaculture Study Programme at Tadulako University and other Sea Partnership members. Results to date have shown that there is still much to learn about this fascinating species including the influence of environmental factors on growth patterns.

(v) Captive breeding research (*ex-situ*) at the Ambon Mariculture Research Station, begun in 2008.

(vi) Monitoring of the trade in *P. kauderni* by the Fisheries Resources Directorate in partnership with the District Fisheries Service, begun in 2008.

(vii) Survey/monitoring of *P. kauderni* populations, planned during 2009/2010.

(vii) Training for fishers in partnership with the Marine Aquarium Council (MAC), possibly in 2009.

(viii) Distribution of a Children's book by Yayasan Palu Hijau with support from PADI Project AWARE, the title means "The little beauty in the sea urchins".

Conclusion: Quo Vadis, BCF?

Since local awareness first began to develop in late 2004 real progress has been made, and the processes associated with the CITES proposal certainly helped raise the profile of *P. kauderni* at local and national levels. However the fact that the Banggai cardinalfish is now listed on the IUCN "Red List" as "Endangered" is a reminder that there is still a long way to go to fulfil the goal of the Banggai Cardinalfish Action Plan, which roughly translates as ensuring the conservation of the species and it's habitat for the benefit of present and future generations. As a key element of the conservation strategy there is a realistic chance that a sustainable ornamental fishery will be developed for this attractive reef-associated fish with an unusual lifecycle whose beauty has nearly been its downfall.

Acknowledgements
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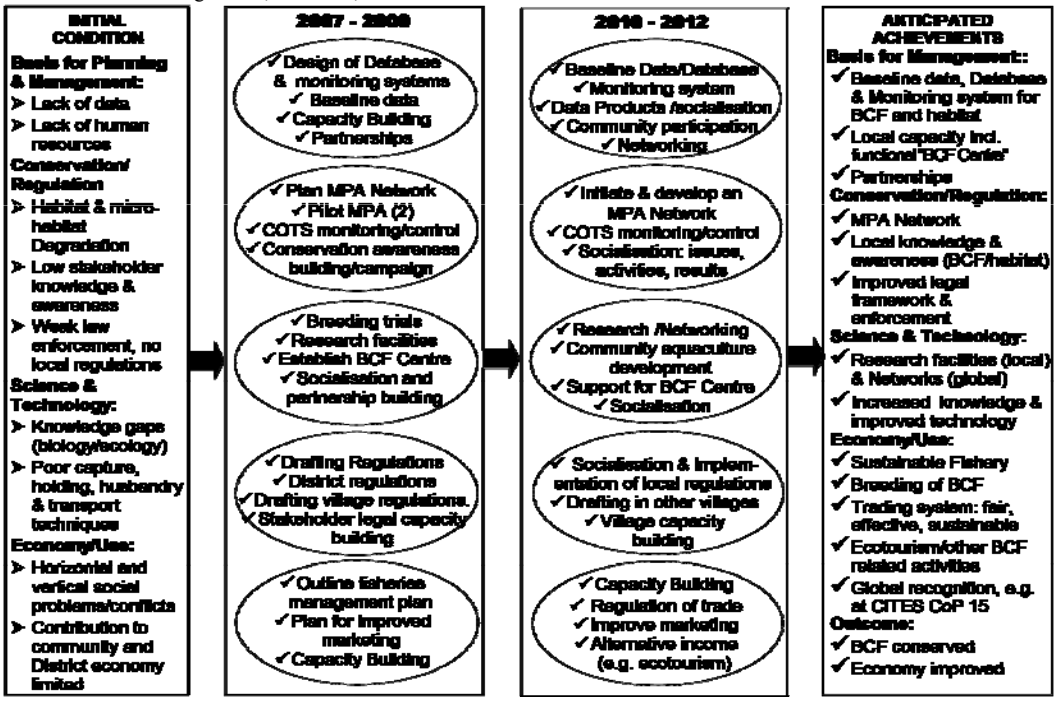

Figure 3: Outline of the Banggai cardinalfish Action Plan.

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Differential depth effects upon biomass patterns in an herbivorous coral reef fish assemblage.

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Abstract. The abundance of parrotfish and surgeonfish on fore-reef habitat was sampled on 6 reefs along an inshore-offshore gradient in La Parguera, Puerto Rico to provide for within reef (across depths) and among reef (cross-shelf) comparisons. Temporally replicated visual surveys were conducted along permanent belt transects ($100m^2$) at three depth intervals (3, 10, 15 m) to obtain data on fish species density and lengths used to calculate biomass. The herbivorous fish assemblage was dominated by small-bodied parrotfish *Scarus iseri*, *Sparisoma aurofrenatum S. viride* and *Acanthurus coerulus*. Overall the density and biomass of parrotfish and surgeonfish was higher at 3m than at 10 or 15m. However, the differences in biomass across the depth gradient were most notable at inshore reefs where the proportional decrease in biomass by depth was higher compared to the mid-shelf reefs. Depth is a well know driver of herbivorous fish abundance, but along a cross-shelf gradient additional within reef factors interact to structure biomass patterns.
Key words: Reef fish, grazing, depth gradient.

Introduction

Grazing by Parrotfish (*Scaridae*) and Surgeonfish (*Acanthuridae*) is an ecological process that structures coral reef benthic communities (Hughes 1994; Belliveau and Paul 2002). Spatial variation in the abundance of these herbivores can result in differential grazing pressure and may have implications to coral-algal interactions (Lewis and Wainwright 1985; Mumby et al. 2006). At a basic research level the patterns in fish distribution on a reef may indicate underlying processes that influence how fish use available habitats. At an applicable level understanding the processes that structure the abundance of parrotfish and surgeonfish will aid in managing for this important ecological function. Understanding the spatial patterns of parrotfish and surgeonfish on fore-reef habitats along an inshore-offshore gradient will help determine factors that structure this fish assemblage (Williams and Polunin 2001; Paddack et al. 2006; Fox and Bellwood 2007).

This study investigates spatial patterns of parrotfish and surgeonfish biomass on fore-reef habitats across the seascape asking: What area the relative abundances of the species from these two families on the fore-reefs of Southwest Puerto Rico? How are parrotfish and surgeonfish distributed spatially across the fore-reef zones in terms of density and biomass? Are the patterns in abundance consistent between the inner-shelf and mid-shelf reefs?

Material and Methods

Fish abundances were sampled on 6 reefs on inner-shelf and mid-shelf reefs to allow within reef (across depths) and among reef (cross-shelf) comparisons (Fig. 1).

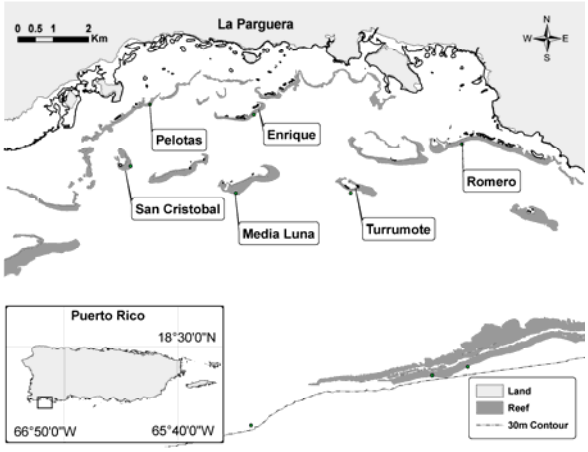

Figure 1: Map of the 6 study reefs (3 inner-shelf: Pelotas, Enrique, Romero; and 3 mid-shelf: San Cristobal, Media Luna, Turrumote).

Visual surveys were conducted along permanent belt transects (25 x 4m). Three depth intervals were selected on the fore-reef slope (3, 10, 15 m), and 3 replicates per depth were sampled 4 times per year. Fish species counts and fork lengths were recorded for each transect. Length data were used to calculate mean sizes and biomass was derived from published length-weight relationships (Ref). Data were analyzed for patterns across depths and between inner and mid-shelf strata by species and at the family level. Kruskal-Wallis (KW) non-parametric ANOVA was used to test for significant differences.

Results

The assemblage of roving herbivorous fish on the fore-reef was dominated numerically by small bodied species. The parrotfish were principally represented by 3 species: *Sparisoma aurofrenatum*, *S. viride* and *Scarus iseri* with the highest densities. Large bodied Scaridae such as *S. guacamia* and *S. vetula* were present but at very low densities and these were generally sighted on the same study reefs. Three species of surgeonfish are present and *Acanthurus coerulus* was numerically dominant.

Across all reefs the abundances of both groups were distinctly structured in relation to depth in the fore-reef habitat. Both families exhibited the highest densities and biomass in the shallowest depth interval (3 m) which sharply decreased at the 10 and 15m intervals (Fig. 2, 3). Densities across depth intervals were significantly different for parrotfish (KW, p=0.036) as well as for surgeonfish (KW, p=0.000). Biomass for parrotfish was 2.3 times higher at the 5 m depth interval than the 15 m interval, and for surgeonfish it was 4 times higher.

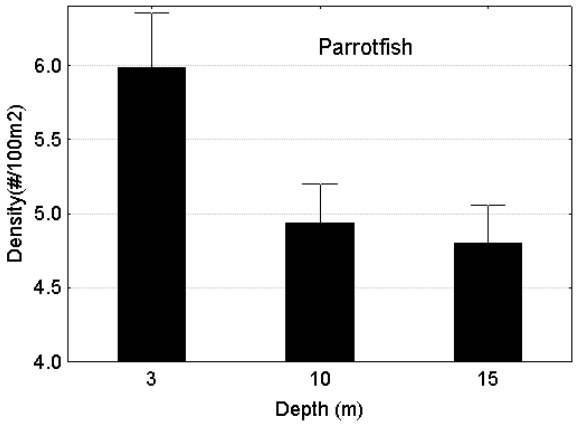

Figure 2: The overall mean density (± SE) of all parrotfish at the 3 depth intervals for the 6 study reefs.

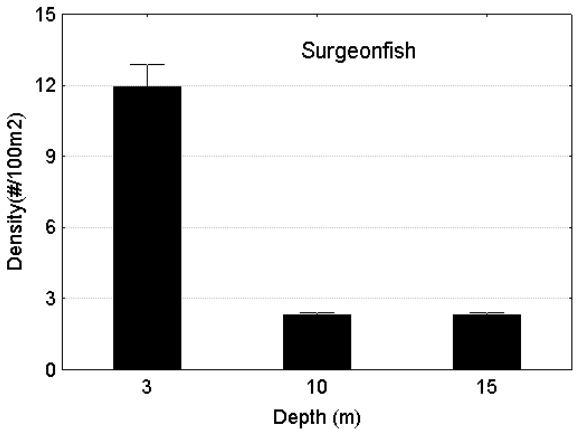

Figure 3: The overall mean density (± SE) of all surgeonfish at the 3 depth intervals for the 6 study reefs.

Comparing the biomass by depth of both families between inner-shelf and mid-shelf reefs revealed that the general trend of decreasing abundances with depth varies between the two strata. The depth-related decrease in abundance is more pronounced at inner-shelf reefs. Biomass for parrotfish on inner-shelf reefs at 3 m was 3.2 times greater than at 15 m; on mid-shelf reefs the proportional difference was 1.8 times greater (Fig. 4). However, comparing the mean biomass of parrotfish between inner and mid-shelf strata for the 3 depth intervals revealed that only the 10m depth interval was significantly different (KW, p=0.003).

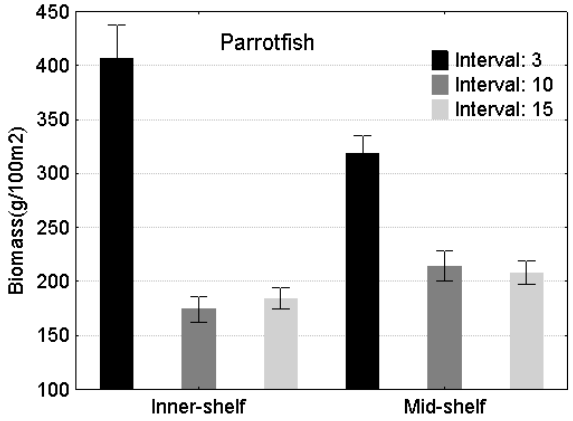

Figure 4: The mean biomass of parrotfish at the 3 depth intervals for inner and mid-shelf reefs, with 95% confidence intervals.

For surgeonfish, biomass on inner-shelf reefs at 3 m was 10.9 times greater than at 15 m; on mid-shelf reefs the proportional difference was 2.8 times greater (Fig. 5). Comparing the mean biomass of surgeonfish between inner and mid-shelf strata for the 3 depth intervals revealed that only the 15m depth intervals were significantly different (KW, p=0.000).

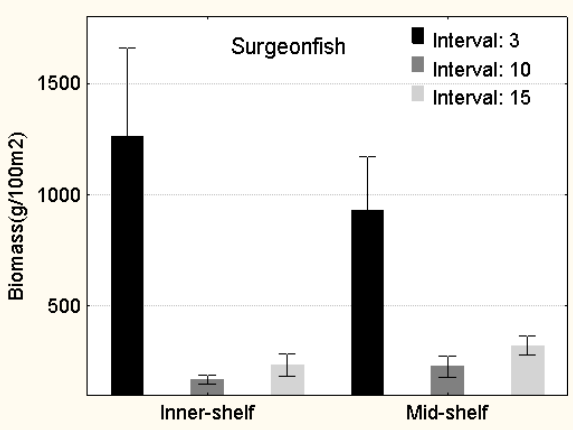

Figure 5: The mean biomass of surgeonfish at the 3 depth intervals for inner and mid-shelf reefs, with 95% confidence intervals.

Discussion

Herbivory is an ecological process that structures coral reef benthic communities and variations in the spatial distribution of herbivores may reflect differential grazing pressure (Mumby et al. 2006). Depth appears to be a major factor that structures the way that parrotfish and surgeonfish utilize fore-reef habitats as has been found in other studies on coral reefs world-wide (Lewis and Wainwright 1985; Hoey and Bellwood 2007).

In this study both the parrotfish and surgeonfish biomass decreased with depth and this was proportionally greater at inner shelf reefs. This effect can vary with reef location, possibly due to factors which are also related to distance from shore, such as water quality (Clifton 1995). Deeper fore-reef habitats seem to be less favorable to grazers which was shown by lower densities and biomass at the deeper depth intervals. This pattern was more pronounced at inshore reefs. These results are in agreement with other studies that have found a peak in herbivorous fish densities in shallow fore-reef zones (Russ 2003; Fox and Bellwood 2007). Maximum abundance at the shallow fore-reef is a result common to most studies on parrotfish and surgeonfish. This may be due to preferential food resources being available from higher productivity of algae at shallower depths (Russ 2003). However, this study documents that this pattern can vary among reefs across an inshore to offshore gradient. The fact that the use of fore-reefs by the dominant species of herbivores is site specific has implications for management strategies that aim to promote fish grazing.

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Photo-ID on reef fish – avoiding tagging-induced biases

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Abstract. Mark-recapture experiments are essential to estimate population parameters. However, physical tagging is often biased, potentially affecting mortality rates, performance, and behavior. Photo-ID is an unbiased alternative commonly used on marine mammals, but has barely been used on teleost fishes. This study examined the individuality and stability of coloration patterns of two rabbitfish species (*Siganus javus* and *Siganus guttatus*) to determine if photo-ID could be extended to teleost fish. Examination of 189 *S. javus* and 34 *S. guttatus* revealed no duplicated coloration pattern within both species. The coloration patterns of six sub-adult *S. javus* and four adult *S. guttatus* remained easily recognizable over two and eight months, respectively. The results of this study suggest that the coloration patterns of *S. javus* and *S. guttatus* are a reliable means to recognize individuals over time, thus allowing unbiased mark-recapture studies on two coral reef fish species.

Key words: photo-ID, *Siganus javus*, *Siganus guttatus*, coloration pattern

Introduction

Assessing the status of populations and managing them as resources heavily rely on the knowledge of population size and parameters influencing it, namely the BIDE parameters (birth, immigration, death, and emigration; Williams et al. 2002). Many of these parameters can be determined via mark-recapture experiments (Amstrup et al. 2005). However, physically marking animals with tags may have negative impacts on the parameter to be estimated, affecting the reliability of the results (Thorsteinsson 2002). For example, stress due to capture and handling, infection risks, and changed performance may increase post-tagging mortality and therefore bias estimated survival rates (Thorsteinsson 2002).

Mark-recapture experiments using photographic identification (photo-ID) forego tagging induced biases (Amstrup et al. 2005). This technique determines encounter histories by recognizing animals based on naturally occurring morphological features, such as scars, shapes, or coloration patterns (e.g. Speed et al., 2007; van Tienhoven et al., 2007), thus avoiding physical tagging altogether.

This approach has two major underlying assumptions: 1) every individual of a population is sufficiently different from other individuals that they are not misidentified; and 2) features or coloration patterns do not change over the time scale of the study.

It is generally accepted by the research community that no individual is completely identical to its conspecifics. Very important theories are based on such individual variation (e.g. Darwin's evolutionary theory; Campbell, 1995). However, phenotypic differences can be subtle. In practical terms for photo-ID, two coloration patterns could be similar enough to appear identical to an observer, thus leading to misidentifications.

In contrast to generally accepted individuality, the stability of coloration patterns is a major concern using photo-ID. In order to reliably identify an animal, the pattern should not change over the time course of the study. In many fish species, this can potentially pose a problem: Ontogenetic shifts, mood changes as well as growth can induce changes in the coloration pattern (Allen 2002). Alterations due to mood changes are usually very obvious and of little impact to mark-recapture studies.

More important are gradual changes associated with the growth of an animal. Patterns do not necessarily grow in proportion with the body. Gradually expanding patterns by the addition of marks or splitting of dots or lines is also possible. Leopard sharks (*Stegostoma fasciatum*), for example, loose their stripes by dissolving them into spots and rings while growing (Mahon J. L., pers. comm.). Because this is normally a slow and gradual process, it is easily overlooked and therefore may pose a major challenge in photo-ID. Over time, the coloration pattern of an individual might change enough such that it is no longer identified as the same animal.

Photo-ID has been successfully applied on many large and long-lived species, typically marine mammals (e.g. Caswell et al., 1999; Neumann et al. 2002; Langtimm et al., 2004). However, application of photo-ID is by no means restricted to such species.

For example, with their highly ornamented coloration patterns many coral reef fishes are good candidates for photo-ID. Nonetheless, this non-intrusive technique has barely been applied to teleost fish, and data that is unbiased by physical tagging is rare.

This study aimed to extend photo-ID as a reliable technique to two herbivorous fish species on coral reefs: *Siganus javus* and *Siganus guttatus*. The coloration of members from both species was examined over time to establish the temporal extent to which the coloration patterns of these two species could be used as reliable identifiers. Furthermore, patterns were also examined to determine their use for individual identification, and to determine the frequency of coloration duplication. The right and left sides of individuals were also compared to confirm the absence of symmetry.

Materials & Methods

Individuality

A total of 189 *S. javus* and 34 *S. guttatus* were photographed on their right and left sides. Photographs were taken approximately perpendicular to the side of the animal, both from live and dead fish. Live animals were photographed in a small aquarium with little interference. The shape and relative position of dots and lines on the entire body of the fish (excluding fins and head) within each species and side were manually compared with each other. The right and left sides of each individual were also compared with each other.

Comparisons were classified as 'identical', 'different', or 'undetermined'. Two photographs were defined as 'different' if three or more differences in coloration pattern could be detected. Instances where photograph quality, reflections, or abrasions prevented comparison over more than approximately 75% of the fish body were classified as 'undetermined'.

Stability

A school of sub-adult *S. javus* was held in the quarantine area of Underwater World Singapore (UWS) and was monitored over time. Throughout the study, a total of 12 animals were fin-clipped in different combinations in the posterior part of the anal, dorsal, and caudal fins for individual recognition within a common tank. The total length of each fish was also measured. In contrast, only four adult *S. guttatus* were available in a large exhibit at UWS. All photographs were taken with minimal interference, i.e. by patiently waiting outside the tank for the animals to swim by. Only photographs approximately at a right angle from the body surface were selected.

Comparing two photographs from the same side and individual, the number of changes was recorded. Splits, fusions, appearances, or disappearances of dots or lines were all treated identically. Right-sided and left-sided photographs were analyzed separately.

Siganus javus

Changes over hours: Seven animals were monitored over eight hours of a day to check for short-term changes in coloration patterns. They were photographed on both sides within 30 minutes, at 8am, 10am, 12noon, 2pm, and 4pm. This experiment was repeated on four days (September 19, October 17 and 24, and November 7, 2007). Photographs were manually compared to detect any changes over two-, four-, six-, and eight-hour intervals.

Changes over days: Similarly, photographs from 23 days were used to examine the variation in coloration patterns of 12 animals over one to six days.

Changes over weeks: Photographs from October 24, 2007 were compared to photographs taken one, two, three, four, and five weeks later. A total of 11 fish were monitored in this way.

Assuming that changes in coloration patterns occur gradually and at a constant rate over weeks, averaging changes over several consecutive equal time periods is more representative for a particular side of a fish. For the different weekly intervals (i.e. one- to five-weekly), non-overlapping intervals were compared and averaged to represent the rate of change.

Long-term changes: Photographs of six fish on each side were available for comparisons over the two months from September 27, 2007 to November 28, 2007 (62 days). Only a single fish was monitored over more than two months. The photo series of this animal covered 161 and 168 days on the right and left side, respectively. It was used to track changes and document their appearance on a time scale.

Siganus guttatus

Four *S. guttatus* were photographed at irregular time intervals from September 4, 2007 to May 5, 2008 (244 days; approximately 8 months). The best photographs taken on the first and last day of observation were compared to determine changes that may have appeared on each side of each fish. The photo series were examined to determine the first appearance of each change.

Statistical analysis

Working with count data, errors are not normally distributed and therefore, two samples were compared with the non-parametric Wilcoxon rank sum test (Crawley 2005). Program R was used to conduct the analysis (The R Foundation for Statistical Computing 2007).

Results

Individuality

The relation of six (0.03%) and nine (0.05%) comparisons of *S. javus* on the right and left side, respectively, could not be determined due to photograph quality, reflections or abrasions. No comparison resulted in an 'identical' result (Fig. 1). Every comparison of *S. guttatus* was found to be different (Fig. 1). All 189 individual *S. javus* and all 34 individual *S. guttatus* differed in their coloration patterns on their right and left sides.

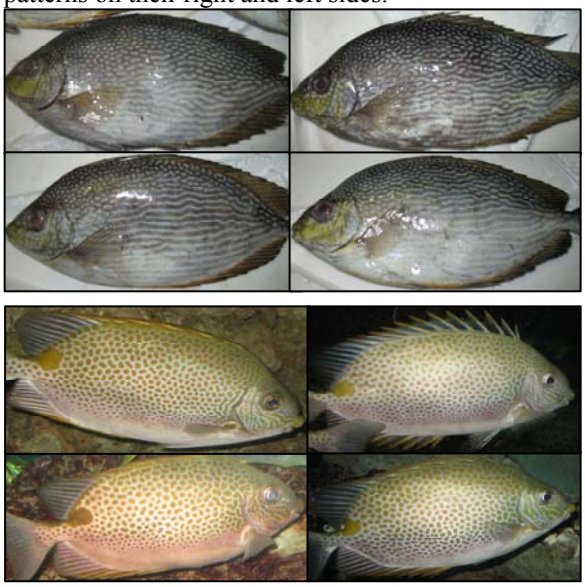

Figure 1: Examples for distinctively different coloration patterns of *S. javus* (top) and *S. guttatus* (bottom). Differences in *S. javus* were most evident at the posterior part below the dorsal fin, while differences in *S. guttatus* were more distinctive behind the gill cover.

Stability

Changes in coloration pattern occurred gradually over time (Fig. 2). They occurred faster in sub-adult *S. javus* than in adult *S. guttatus*.

Siganus javus

Fish were sized from 14 cm to 19 cm at the time of fin-clipping. The first differences in coloration patterns of *S. javus* occurred in weekly intervals. No changes were observed within time intervals of less than one week.

Changes over weeks: Within all one- to five-weekly intervals only three out of 12 fish showed some changes. Over one-, two-, and three-weekly intervals no more than a single change per side and fish was observed. A maximum of two changes per side and per fish was found in four- and five-weekly intervals.

The number of changes observed on the right and left sides were not significantly different ($p > 0.3404$). Single measurements from the same date and averaged measurements from consecutive intervals showed similar results for one- to five-weekly periods ($p > 0.1675$). The number of changes was not significantly different from zero ($p > 0.3458$).

Long-term changes: Over two months, only two out of six fish accounted for all eight changes (one fish had two changes per side, the other one showed four changes on the right side). The numbers of changes were not significantly different from zero ($p > 0.3711$), and did not differ between the right and the left sides ($p = 0.5271$).

Over longer intervals, 16 differences on the right side over 161 days and 13 differences on the left side over 168 days were recorded from a single *S. javus* monitored. Averaged over approximately 5.5 months, this corresponded to a monthly changing rate of 2.9 and 2.4 changes per month (CPM) on the right and left sides, respectively. Changes gradually accumulated over time.

Despite the changes, individual *S. javus* could easily be recognized after two months, including the side of one fish that underwent four changes. However, after 161 and 168 days (app. 5.5 months) on the right and left sides, respectively, discrepancies made recognition less easy, i.e. manual matching would most likely have failed on the right side.

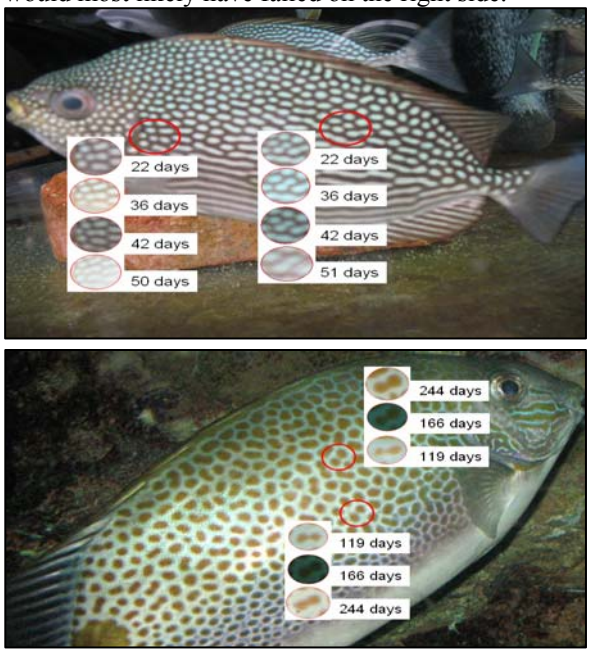

Figure 2: Gradual splitting dots in *S. javus* (top) and *S. guttatus* (bottom). The number next to the circles indicates how many days earlier the picture of this section was taken. Note that the splitting process was much faster in the sub-adult *S. javus* compared to the adult *S. guttatus*.

Siganus guttatus

On the right and left side, the four animals experienced on average 3.5 and 2.75 changes, respectively, over the 244-day observation period.

This was equivalent to 0.4 and 0.3 CPM. Early in the observation period, one fish obtained a superficial scratch on the right side, which healed in the time course of the study. The pattern at the location of the scratch remained largely unchanged. Only one dot stretched into a short line. Over the entire period, all four animals could very easily and unequivocally be recognized through photographs of both sides. The number of changes on the right and left sides were not significantly different ($p = 1$).

Discussion

Coloration patterns as individual identifiers

According to intuitive expectations, neither *S. javus* nor *S. guttatus* showed symmetrical coloration patterns. Consequently, photographs taken from the right and left side of an individual failed to match and the two datasets had to be treated separately in mark-recapture studies. Schwarz C. J. (pers. comm.) suggested treating the two datasets as independent sampling methods of the same population.

With no similar coloration pattern found twice within both sides of both species, the data suggests that twin-patterns can be practically excluded. This supports the intuitive assumption taken by many researchers when applying photo-ID: every animal has unique coloration, markings, and features. However, results should not be over-weighted. These schooling fish species occur in large parts of South-East Asia (*S. guttatus*) and, for *S. javus*, stretches as far as Australia and the Persian Gulf (Woodland, 1990). The sample sizes of 34 and 189 specimens of *S. guttatus* and *S. javus* used in this study, respectively, make up a very small sample to consider. Nevertheless, it seems reasonable to assume that twin-patterns do not occur in significant numbers in populations of *S. javus* and *S. guttatus*.

Stability of coloration patterns

Intuitively, one would anticipate coloration patterns to change gradually in relation to growth rates, i.e. patterns are expected to change equally on both sides of the animal and to do so mostly during fast growing stages of the life cycle. Stages with little or no growth are believed to exhibit very stable coloration patterns.

The results of this study support this intuitive theory. Changes occurred gradually and in equal quantities on the right and left side in both species. Assuming that *S. javus* and *S. guttatus* both follow the Von Bertalanffy growth curve, which a majority of marine organisms do (e.g. many elasmobranches; Cailliet et al., 1992; van Dykhuizen and Mollet, 1992; Acanthuridae; Choat and Axe, 1996; Scaridae; Choat et al., 1996), juveniles and sub-adults would be expected to grow fast compared to adults. If changing rates are indeed positively correlated to growth rates, coloration patterns would be expected to also change faster in small fish. According to Woodland (1990), *S. javus* commonly measure less than 30 cm standard length (SL), but they can grow considerably larger. With a maximum of 19 cm total length (TL), the fish used in this study are thus believed to reflect the sub-adult, fast-changing part of the life cycle.

In contrast, the four *S. guttatus* at UWS were fully-grown adults. They have been kept for at least one year prior to the start of the observations (pers. obs.). Although no measurement could be taken, animals were estimated to be close to 30 cm SL in July 2007. With a maximal SL of 35 cm reported by Woodland (1990), it is reasonable to assume that the animals at UWS represent the slow-growing and thus, the slow-changing phase of their life cycle.

In accordance with the intuitive theory, the sub-adult *S. javus* monitored over more than five months showed a much larger change in coloration pattern than did *S. guttatus* over eight months. With 2.9 changes per month (CPM) on the right side and 2.4 CPM on the left side *S. javus* changed at a much faster rate than *S. guttatus*, which averaged 0.4 and 0.3 CPM on the right and left side, respectively. After five months, *S. javus* could no longer be unambiguously identified, while *S. guttatus* were easily recognized even after eight months.

Although growth pattern and changing rates are not generally comparable among different species, they can be assumed to be similar in the two species of interest here. Both species show many anatomical similarities such as spine arrangement, jaw structure, and fright pattern (Woodland, 1990). They both grow to a comparable size, and have similar patterns consisting of dots and lines (Woodland, 1990). Furthermore, they are genetically very closely related (Lemer et al. 2006).

The results from sub-adult *S. javus* suggest that patterns only remain entirely stable on a very short time scale. After six days, changes started to occur in some fish, but animals remained easily recognizable even after two months, with up to four discrepancies per side. Therefore, photo-ID can be reliably used on *S. javus* up to 62 days. The fact that the number of changes was not significantly different from zero over all time intervals up to two months supports this conclusion.

The two-month reliability for photo-ID of sub-adult *S. javus* is a conservative estimation. The single fish monitored for a longer period showed only six changes after 134 days on the left side. Over both intervals, it was still possible to identify the two photographs as taken from the same individual, suggesting that photo-ID could also be used over time periods of up to four months. Likewise, the right side

remained easily identifiable over 127 days despite 13 changes. Identification of individuals on both sides only became difficult after more than a five-month interval.

In contrast, the adult *S. guttatus* exhibited much more stable coloration patterns. After the entire observation period of 244 days (app. 8 months), only very few changes occurred. All animals remained unmistakably identifiable. Thus, mark-recapture studies using photo-ID can be reliably used on adult *S. guttatus* over at least eight months.

To apply these results mechanically to all populations of *S. javus* and *S. guttatus* and its members may lead to inaccuracies. The two-month stability observed in sub-adults of *S. javus* is likely to be a more accurate estimate for sub-adult *S. guttatus* than the eight-month stability determined for adult *S. guttatus*. Identification of a sub-adult, presumably fast-growing *S. guttatus* after eight months is likely to fail and thus may lead to unrepresentative data. Similarly, adult *S. javus* may exhibit a stable pattern similar to that of adult *S. guttatus*.

The reliable identification intervals found in this study contrast with conventional tagging. The latter is often marked by a high rate of tag loss and tagging induced mortality shortly after release, followed by a period of lower loss rates (Thorsteinsson 2002). In contrast, coloration patterns are not lost and change only gradually. Thus, photo-ID studies are most reliable directly after the initial photographic capture and can potentially generate more accurate data in this regard.

Extraction of encounter histories from photographs is a tedious process, a disadvantage compared to physical tagging. However, the use of new software can accelerate the comparison of photographs (Speed et al. 2007; van Tienhoven et al. 2007). Furthermore, photo-ID is preferable when capturing live animals is difficult or when mortality rates have to be kept at zero (e.g. for species at risk).

While it is relatively easy to conduct photo-ID studies in captivity, obtaining photographs in the wild may pose a problem for non territorial and schooling fishes. However, photo-ID was still successfully used by the author to estimate short-term site-fidelity and localized population sizes of *S. guttatus* and *S. javus*. Territorial non schooling fishes (i.e. many Serranidae) promise more extensive applications of photo-ID.

In conclusion, the data suggests that photographs of individual sub-adult *S. javus* and adult *S. guttatus* can be reliably recognized over at least two and eight months, respectively. Within these conservative limits, photo-ID is believed to yield very accurate results for the two species: *S. javus* and *S. guttatus*.

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Management of the aquarium fishery in the Republic of the Maldives

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Abstract. The marine aquarium trade of Maldives started around 1979 when most of the exports were sent to Sri Lanka. As the fishery matured, Europe became the major destination as the earnings related to quantity shipped increased. In 2007, there were seven registered companies, trading approximately 140 species of fish and 5 species of invertebrates. The contribution of the marine aquarium trade to the total value of marine exports is 0.55% and worth US$590,530 in 2007. The Maldives is a popular destination for snorkelers and divers and the collection of aquarium could impact dive tourism. Presently, a species based quota system is in effect that is derived from export data from the Maldives Customs Services, knowledge of the abundance of each species and knowledge of its tolerance to captivity. Random checks of the fish exports are scheduled for implementation following the introduction of a fish guide software. There are no specifically designated areas for collection of aquarium fish and the collection moved from one area to another as stocks declined. This pattern of resource use may act as indirect time area closures and aid in the replenishment of fish stocks. The last aquarium fish stock assessment survey was performed this year.

Key words: Economic value, ornamental fish trade, MPA

Introduction

The marine aquarium trade of Maldives is believed to have started around 1979 (Adam 1996). During the early years most exports were to Sri Lanka (Edwards and Shepherd 1992), and over the years the fishery has expanded, Europe becoming the major destination. In 2007, there were 7 registered companies, trading a total of about 140 species of fish and 5 species of invertebrates. Export of corals is banned in the Maldives and thus the trade does not include coral species. The only exception to this ban is the export of organ pipe coral (*Tubipora musica*), a species which is not traded in the aquarium fishery but nonetheless exported for pharmaceutical purposes, mainly to India.

Only a few species in the aquarium trade are directly exploited for other purposes, and aquarium species are probably the highest value-added product that is collected from coral reefs (Wabnitz et al. 2003). In 2007, 358,378 fish and invertebrates were exported from the Maldives, earning a total of Rf 7,529,266 (US$590,530) and making up 0.55% of total value of marine exports. Although the numbers of fish taken are relatively small, there is a potential for conflict of interest between the fishery and tourism, the major source of foreign exchange earnings. Tourism in the Maldives is essentially marine based and therefore heavily dependent on the rich diversity of this environment. The species that are collected by the aquarium trade are also an important attraction for tourists who visit the Maldives. As collection of fish is allowed on many of the tourist dive sites, there is the possibility of the two activities occurring simultaneously, thus increasing the conflict. Until recently, a few minor bait species were collected for the aquarium trade. Live baitfish is required by the pole and line tuna fishery, the primary fishery of Maldives. Juveniles of some species of edible reef fish such as *Variola louti* (Lunar tailed grouper) and *Cephalopholis miniata* (Vermillion rock cod) which are eaten locally or targets of the live food fish trade are also collected by the aquarium fishery. However, the quantity exported through this trade is insignificant compared to the amount landed at the fish market or exported as live food fish. For example, 23 juveniles of *C. miniata* and none of *V. louti* were exported in the aquarium trade between May and September of 2003. During the same period, 11,888 individuals of *C. miniata* and 12,992 of *V. louti* were exported in the live food fish trade (MRC unpublished data, 2003).

Due to such matters, along with concern of potential ecological and environmental impacts arising from the fishery, the Government of Maldives has closely monitored the trade since its inception (Adam 1996). In 1988, a blanket quota of 100,000 was set for maximum total number of exports of all species of fishes by Maldives Customs Services in consultation with the Marine Research Section (currently named Marine Research Centre) (Edwards 1988). While such

a quota system is simple and could be easily enforced, it could also lead to overexploitation of sought after species even when total exports are below the blanket-quota level, especially since about 20 species makes up about 70% of the trade (Edwards and Shepherd 1992). Thus, a provisional species based quota system was adopted for 22 species (Edwards and Shepherd 1992) but adherence and enforcement was lacking. More recently a species-based quota system for the majority of the species traded has been implemented. This has strengthened monitoring of the trade and aided management of the fishery.

Material and Methods

The data for this report was obtained from export information collected by Maldives Customs Services (MCS). The quantity of fish exported and the revenue derived each year is compiled by MCS. The trend in exported quantity and value of exports was obtained using the MCS information. The species data was compiled from 286 proforma sheets filled out by the exporters. This data set was not complete but gave quite a clear representation of the total exports.

Interviews were carried out with managerial staff of the two major exporting companies to investigate the processes involved in the trade, management issues and to identify difficulties with data collection. Information on the process of fish and invertebrate collection was gathered during a field visit to the aquarium of the largest exporting company, Sub Tropical Exotic Paragon (STEP) in 2000 and subsequently in 2004 and 2008. Fish collectors and staff of the aquarium were interviewed during these visits.

Status of the Trade

Over the years, the fishery fluctuated with Europe becoming the major destination (Figure 1). In 2007, there were seven licensed companies in the aquarium trade. However, 54% of the exports were by one company, earning 66% of the total revenue. Approximately 90 people are employed in the trade of which only a small number are expatriates working in the managerial and administrative positions. This is in contrast to the mid-90s when majority of the work force were Sri Lankans working essentially as divers (Adam 1996). This transformation was probably due to enforcement of Clause 14 of the Fisheries Law (Law No. 5/87) which states that only Maldivians have the right to carry out fishing activities in the fishing grounds most commonly used by Maldivian fishermen, which represents an area within 75 miles of the outer atoll rim. This was triggered as a consequence of the increasing use of moxy nets, a destructive method of fishing which was introduced to the fishery by the Sri Lankan divers (Adam 1996).

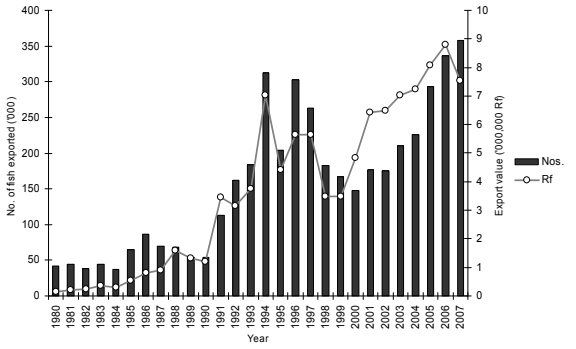

Figure 1: Trends in the export of aquarium fish from 1980 – 2007 showing the number of individuals exported and revenue earned. Source: ERSS/MoFAMR and MCS.

Species Based Quotas

The species-based quota system that is in effect at present was based on MCS export data and on general information of species such as rarity in the natural environment, as well as tolerance of species to captivity. Such a selective quota system is difficult to establish, as information on population characteristics such as abundance and boundaries of habitats in collection areas and species densities is required to understand maximum sustainable yields, and to identify those species that are susceptible to overexploitation.

There are three categories adopted in this system. Category A includes 17 individual species that are banned from export. Category B includes 66 species that are subject to an export quota. Category A includes species that do not survive well in captivity such as *Chaetodon meyeri, C. trifasciatus* and *C. triangulum* (Wood 1985) and those that are locally rare like the Armitage's angelfish (*Apolemichthys armitagei*). In addition to Category A listed species, it is an offence to export any species of parrot fishes, puffer fishes, porcupine fishes, eels or giant clams. Category B include species from families Pomacanthidae, Ephippidae, Ostraciidae, several Chaetodontidae and Pomacentridae, Cirrithidae, Monacanthidae, Serranidae, several Labridae, Balistidae, Plesiopidae and Scorpaenidae. Category C which consist of 71 species that can be freely exported up to a maximum total number of 300,000 individuals. There is also a ban on exporting any species utilized as live bait in the pole and line tuna fishery. In compliance with this regulation, the most traded species globally, *Chromis viridis* (Blue-green damselfish) (Wabnitz et al. 2003), is not exported from Maldives.

The current system appears to be quite effective, although there are still some difficulties in monitoring the trade. The system was administered through the use of proforma aquarium fish export forms as

recommended by Edwards and Shepherd (1992). The form lists species' scientific and common names that are commonly exported in the trade. It also allocates a code name to each species and the exporters fill out one proforma sheet per consignment. The problem with this system lies in effectively monitoring the quotas as there are different agencies involved in the process. While the Marine Research Centre (MRC) sets the quotas, Ministry of Economic Development and Trade (MTI) issues export licenses and MCS collects the proforma sheets filled out by the exporting companies. MCS compiles data summaries such as quantity of fish and revenue from each consignment as well as the destination and dates.

Copies of these sheets are forwarded to MRC where the species data is compiled. Thus clarity of responsibilities with regards to monitoring quota balances is lacking. As a result, every year a few species in Category B are exported in access of the quotas set for these species. Although infrequent, a few individuals in Category A are also exported occasionally.

Another problem is the use of a wide variety of common names to identify the different species. It is not clear how reliable the data collected from the proforma export forms is. One of the exporting companies reported that they were not very familiar with the species names and therefore were not very confident when filling out the proforma sheets.

Nonetheless, in the absence of species catch and effort data, the species based quota system provides reliable estimates of the trade including numbers of fish exported, revenue derived from the trade, destinations, species data and unit prices.

Licensing scheme

The mandate to regulate the export trade lies within the Ministry of Economic Development and Trade (MEDT). They are responsible for issuing export licenses to the aquarium fish exporting companies and this license is based on the export value, which is determined by the exporter. The minimum value is Rf 100 (Rf12.75 =US$1.00) and there is no upper limit for the maximum value. There is a 0.1% tax on the value of the exported fishes. The quota for Category B species is determined by MRC for the year and is divided up, and a part is given out under the license. The license is valid until the last day of the year it was issued. As the license is based on the value of the exports, once the license value is reached, it has to be renewed even though some export quota is remaining under that particular license. If a species has been exported up to the quota issued with the license, the quota is renewed provided that the overall yearly quota set for that species by MRC is still remaining.

A major problem with a value based licensing system can be under quoting of prices of fish by exporting companies in an attempt to maximize the number of fish exported per license. This would have consequences on the assessments of the annual revenue from the fishery as well as average unit prices. Thus the present system of licensing should be eliminated and a quota based system put in its place. For this to be achieved, all species traded in the industry need to be given a yearly quota including those in Category C.

Collection areas

Aquarium fish collection can be carried out on any reef except resort house reefs and the 25 protected dive sites which were established in 1995 and 1999. In the beginning, collection areas were concentrated around Male' due to the dependence of the trade on an international airport, with direct flights to Europe and other destinations (Edwards and Shepherd 1992). With the establishment of regional airports in the northern and southern atolls and the introduction of faster boats, collection has spread to other atolls including Baa in the north, Male and Vaavu atolls in the central region and Gaafu Alifu and Gaafu Dhaalu in the south (Fig. 2).

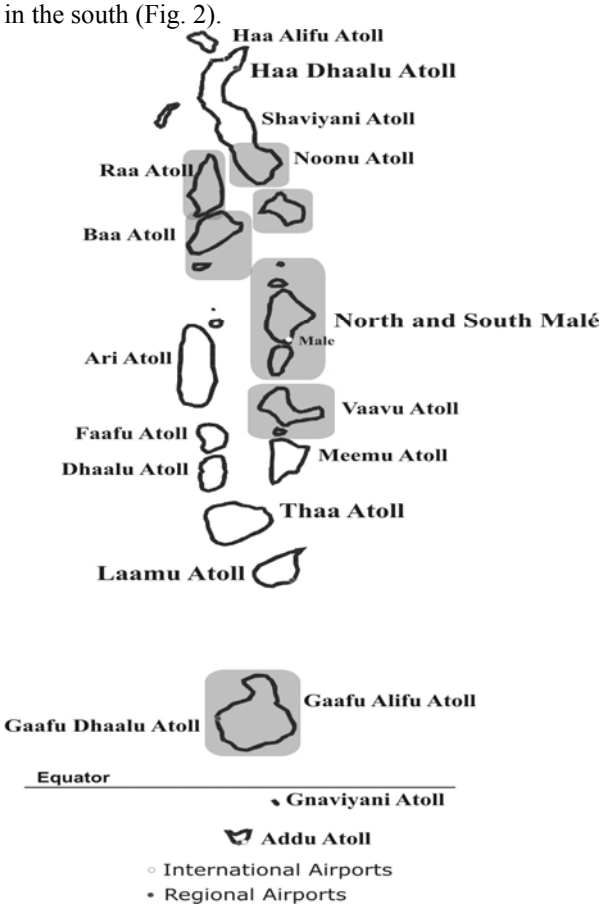

Figure 2: Map of the Maldives showing areas of collection 2008 (shaded areas)

The collected fish are brought by domestic planes and speed boats to the holding facilities in Male' atoll and conditioned before sending them abroad. A maximum of 50 l of fuel is used per day on the boat to get to collection sites and return to base. This suggests that collection is carried out in an estimated area that can be reached within a 1 hr boat ride.

There have been changes in collection areas over time. In 2000, collection was carried out in Haa Dhaalu, Male', Laamu and Thaa atolls while the collection areas were in Baa, Male' and Vaavu atolls in 2004. More recently collection occurs in Noonu, Raa, Baa, Lhaviyani, Male', Vaavu and Gaafu Alif and Gaafu Dhaalu atolls. This pattern of resource use may act as temporary area closures and aid in the replenishment of fish stocks.

At present there are no designated areas for collection of aquarium specimens. From a scientific point of view it is very important to have designated areas for collecting aquarium fish. Edwards and Shepherd (1988) recommends a fishing area of 20% and the remaining 80% closed to fishing, thus acting as marine reserves. Such a system would ensure that a recruiting stock is available to replenish the fished areas, minimizing overexploitation (Palumbi 2003) and extinction of sought after species. In the past few decades, marine reserves have been advocated as a tool to replenish overexploited stocks of species targeted by food fisheries (Russ and Alcala 1996, MacClanahan and Mangi 2000). A study carried out in Hawaii on aquarium fish collection and marine reserves found that aquarium fish increased in newly established Fishery Replenishment Areas (FRAs) while there was a significant decrease in those areas open to collection (Tissot et al. 2002). These two sets of sites were compared to control sites for evaluation of effectiveness of FRAs in management of the aquarium fishery.

Awareness initiatives

A guide to fish identification was prepared by MRC in 1996 to aid those involved in the fishery, including Customs officials, fish collectors and managerial staff. More recently an online fish guide has been developed to aid with fish identification and strengthen monitoring. It is hoped that better informed people will yield more accurate data filled out by the exporters. In addition to the guide, workshops are being held for exporters and MCS officials involved in the trade to increase awareness of the fishery and to provide information on strengthening data collection and monitoring. Workshops introducing the fish guide are being planned for this year and the first of the series was held in September 2008 for the MCS officials. Similar workshops will be held for the exporter and the staff of the Ministry of Economic Development and Trade later in the year.

Licensing and data analysis

An important component of monitoring and regulating the trade is to carry out regular reviews of the data, to assess trends and to assess whether existing levels of exploitation are sustainable. An example is the case of the poison goby and the long nose filefish. Disappearance of these species was not detected until a review was done in 2000, two years after the bleaching event. This could have been avoided if the species data are regularly analysed.

At present, quotas are given out to the exporters by MEDT. The exporters are required to present the relevant license with each consignment. MCS keeps tally of the numbers of each species exported to ensure that fish are not exported beyond the quotas given under that particular license. However, in 2002, eight species in Category B were exported beyond their respective quotas. This can be avoided through frequent analysis of species data from the proforma sheets, which would show if the export numbers are approaching quotas set for the year. MRC could play a larger role in monitoring quotas using data from the proforma sheets. However, the data transfer process between MRC, MEDT and MCS needs to be strengthened to make sure that all information is available to MRC in a timely manner in order to carry out the analysis.

Resource Assessment Survey

A resource assessment survey of selected species in the B Category of the quota list was carried out in June 2008. Impact and control sites were selected in Baa Atoll, North and South Male' Atolls and Vaavu Atoll. Timed swims were employed to estimate abundances of fish species while reef resilience techniques were used to assess the status of the habitat and the benthic environment. Results of the survey are expected to be available in August 2008. This survey was carried out to re-assess the quotas allocated for the different species. As the present quotas were based on information from only Male' atoll and since collection has spread to the outer atolls, it was felt that quotas for some species could be adjusted depending on their resource status.

Recommendations

The following recommendations are based on our observations and analysis:

- Establish designated areas for collection of fish for the aquarium trade
- Increase consistency of names used by exporters, collectors, clients and the government agencies

- Introduce daily log books to obtain information on collection areas, hours spent on collection, mortality of fish
- Develop a code of conduct for fish collectors
- Develop criteria or acceptable standards for holding facilities
- Develop criteria for providing certification to exporters from the government
- Carry out a stock assessment of the species exported in the aquarium trade
- Strengthen monitoring of the exports and regulation of the trade through increasing transparency of responsibilities of the different agencies involved in the trade
- Set quotas for all species traded including those in Category C
- Substitute the present system of value based licensing with quota based licenses.

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Reef Fish Spawning Aggregations in the Bay of Bengal: Awareness and Occurrence

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Abstract. Reef fish spawning aggregations are highly vulnerable to over exploitation. Little research has focused on spawning aggregations in the Bay of Bengal. Interview surveys were conducted among fishers in India, Indonesia, Maldives, Sri Lanka and Thailand in order to determine the level of awareness of spawning aggregations, to identify aggregation sites and species, and to assess fishing pressure on and status of spawning aggregations. Results show low awareness of spawning aggregations throughout the region except in the Maldives where fishers relying on visually aided hook-and-line line fishing reported in-water observation. 91 spawning aggregation sites corroborated by several fishers were identified in four atolls in the Maldives, including those of *Epinephelus fuscoguttatus, E. lanceolatus*, *E. polyphekadion*, *Plectropomus areolatus, P. pessuliferus* and *Lutjanus bohar*. This is the first record of spawning aggregations of *P. pessuliferus.* The spawning aggregations are fished, and the growing grouper fishery will require a precautionary management approach. Increasing awareness among managers and policy makers as well as fishers of the ecological significance and vulnerability of reef fish spawning aggregations remains a high priority.

Key Words: coral reef, reef fish, spawning aggregation, reproduction, Bay of Bengal, South Asia

Introduction

Many species from several families of reef fish spawn in aggregations, including some groupers, snappers, parrotfish and rabbitfish (Claydon 2004, Cornish 2005). A common definition of a spawning aggregation is "a group of conspecific fish gathered for the purpose of spawning with fish densities significantly higher than those found in the area of spawning during the non-reproductive periods" (Domeier and Colin 1997, Colin et al., 2003). Domeier and Colin (1997) propose there are two types of spawning aggregations: 'resident' and 'transient', the former generally occurring within the home range of fish that aggregate frequently and throughout the year. The latter may take place at only a specific time of a month once or several times a year, and fish may migrate hundreds of kilometers to an aggregation site (e.g. Bolden 2000). Transient spawning aggregations may represent up to 100% of a species' reproductive output (Domeier and Colin 1997). The larvae from aggregations may travel far before settling out of the plankton to mature. Thus spawning aggregations can supply seed for fish populations over very large areas.

As spawning aggregation sites and timings tend to be consistent, they are often known to communities with a long history of and high reliance on fishing. While subsistence fishing of spawning aggregations may be sustainable in the long-term, such as has been the case in parts of the Pacific Ocean (Johannes 1981), they can easily become over exploited (Sadovy and Domeier 2005). Persistent fishing may cause spawning aggregations to cease, leading to dramatic declines in a species' population (Sala et al. 2001, Aguilar-Perera 2006). Groupers, many of which aggregate to spawn and are long lived with late maturity, are particularly vulnerable to over-fishing (Sadovy 1996, Dulvy et al 2003). However, with adequate protection, spawning aggregations that have been severely overharvested can recover (Beets and Friedlander 1998). Careful management of reef fish spawning aggregations is thus critical to both the health of fish populations and the coral reef ecosystem, as well as to communities that depend on fishing (Domeier et al 2002, Sadovy and Domeier 2005), and should be, but are rarely, considered in Marine Protected Area (MPA) design (Robinson et al 2008).

There is a limited but growing body of scientific literature on reef fish spawning aggregations, and the Society for the Conservation of Reef Fish Aggregations (SCRFA, www.scrfa.org) maintains an online database of spawning aggregations (Cornish 2005, Sadovy et al. 2008). Reef fish spawning aggregations have been documented to some extent in the Western Indian Ocean (Robinson et al. 2004, Samoilys et al. 2006, Robinson et al. 2008) as well as in Indonesia (e.g. Pet et al. 2005), but little published

information exists on reef fish spawning aggregations in South Asia and around the Bay of Bengal, except some detailed studies from one atoll in the Maldives (Sluka 2001 a,b). Even anecdotal information is scarce. Consequently both fisheries and MPA management in the region remains constrained by a shortage of data as well as awareness among management institutions.

Many species known to form spawning aggregations elsewhere are important resource species around the Bay of Bengal, including several groupers, snappers and trevallies. For example, the Maldives has a well-developed, widespread and unregulated reef fishery serving the tourist resorts in the country. There is also a specific grouper export fishery which although regulated is in decline (Sattar and Adam 2005). A grouper fishery is developing fast in the Lakshadweep Islands, India (Tamelander and Hoon 2008, Arthur 2008), and is already well established in the Andaman Islands and in Aceh, Indonesia. Many resource species in the Gulf of Mannar are threatened by destructive fishing and overfishing (Bakus et al 2000).

In view of critical knowledge gaps with respect to fish spawning aggregations in the Bay of Bengal as well as present reef fishery trends, a survey was conducted to identify potential reef fish spawning aggregation locations, species and timings, and determine levels of knowledge of them among fishers.

Materials and Methods

The study was carried out through interview surveys with fishers. Interviews were conducted by field teams familiar with the target areas and with experience of community based research. Interviewers also received training at a regional workshop prior to the surveys. Key informants in fishing communities were targeted, including fishers using a variety of gears and of varying age and experience. A questionnaire based on guidance from SCRFA (Colin et al 2003) and previous surveys in the Indian Ocean (Robinson et al 2004, Samoilys et al 2006) was used. The questionnaire contained questions on personal information (age, years fishing, gear, areas visited etc.); knowledge of occurrence of reef fish spawning aggregations (have aggregations been observed, what appears to be the reason for aggregating, what are the spatial and temporal characteristics etc.); and what species are known to form spawning aggregations. Pictures, including of fish species and hydrated gonads, were used to accompany the questionnaire. In cases where spawning aggregations were known the interview went on to detail habitat, depth, time (month, lunar phase, time of day), as well as fishing pressure, estimated catches and catch per unit effort. The reliability of interviewees was subjectively assessed based on responses provided, including knowledge of the local environment and fish species as well as years of experience, and rated 'high' or 'low'.

Sites, Time and Sampling Effort

Surveys were carried out between March and October 2007, focusing on important reef areas with known reef fisheries in five countries (Fig. 1):

- Maldives: Baa, Dhaalu, Faafu and Vaavu Atolls (56 interviews)
- India: Agatti and Minicoy in the Lakshadweep Islands (49 interviews), Mandapam and Keezhakkarai coast in the Gulf of Mannar (63 interviews), and informal discussions in the Andaman Islands;
- Indonesia: Weh Island in Aceh Province (60 interviews);
- Sri Lanka: informal discussions with fishers in south western and western reef areas;
- Thiland: Phuket Island, Phuket Province, and Bulon island group, Satun Province (190 interviews).

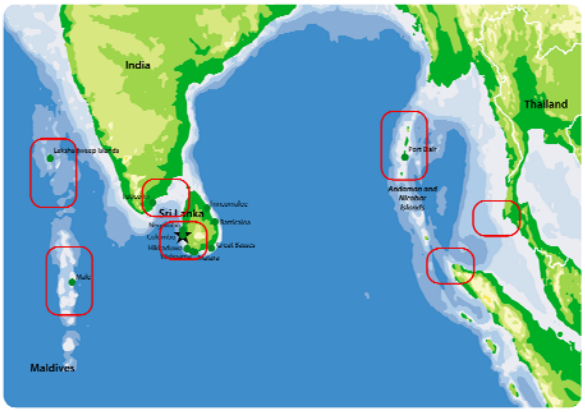

Fig. 1. Map of the region with target areas marked in red.

Data Analysis

Raw interview data were compiled and data from respondents with low reliability ranking and respondents not aware of spawning aggregations were removed. Data from reliable respondents was further queried to confirm potential spawning aggregations reported. Spawning aggregations were considered corroborated if the species and site were mentioned by at least two respondents, or if direct or indirect evidence was furbished, such as observation of spawning or reports of prevalence of hydrated gonads in catch at the time of an aggregation.

Results

Results from the survey are reported here except exact

locations and timings of spawning aggregations, which will not be made public due to the sensitivity of this knowledge. Results will be entered onto the SCRFA online database.

Maldives
Several spawning aggregations were identified in the Maldives, with 91 reported by 2 or more interviewees, 15 of which were reported by 9 or more, and one site reported by 17. Spawning aggregations were validated by reports of sighting of aggregations underwater, and/or enlarged gonads and release of eggs on capture. Fishers reported fish do not take bait when ready to spawn giving low catches during the first few days of an aggregation. .

Spawning aggregations of the following species were confirmed (number of respondents in brackets): *Epinephelus fuscoguttatus* (53)*; Epinephelus lanceolatus* (11); *Epinephelus polyphekadion* (4); *Plectropomus areolatus* (42); *Plectropomus pessuliferus* (11); *Lutjanus bohar* (6). Possible spawning aggregations of *Plectropomus laevis, Variola louti,* and *Caranx melampygus* were reported by one respondent each.

Aggregation depths ranged from several meters to over 50m in *E. fuscoguttatus* and *P. areolatus. E. fuscoguttatus, E. lanceolatus* and *P. areolatus* were reported to aggregate in the 3rd lunar quarter, *Plectropomus pessuliferus* during full moon. Aggregations reportedly tended to be largest during the inter-monsoon periods (April-May and November-December).

India
Fishers at Minicoy and Agatti in the Lakshadweep Islands had no information on reef fish spawning aggregations, although their knowledge of local ecological and environmental conditions is considerable. There were also no indications of spawning aggregations in the near-shore shallow coral areas of the Gulf of Mannar. Fishers reported aggregations of several species 5-10 miles offshore in rocky habitat at depths between 10 and 20m, but while opinions of species, timing, and lunar phase are shared among several fishers from different villages none of the respondents identified spawning as a reason for aggregating. Few interviews were carried out in the Andaman Islands and results are inconclusive. Most fishers were unaware of the phenomenon although there were suggestions of potential spawning aggregations of *Plectropomus* spp. in South Andaman.

Indonesia
Awareness of spawning aggregations was low, and a distinction between spawning and normal schooling was not made. However, although not corroborated, possible spawning aggregations of five species were reported from sites on the west, south and east coast of Weh Island at different times, dependent on location and species: *Bolbometopon muricatum*, *Cephalopholis miniata*, *Variola louti*, *Cheilinus undulatus*, and *Caranx lugubris*.

Sri Lanka
Informal discussions with fishers indicated no awareness of spawning aggregations in southern and western Sri Lanka.

Thailand
Some fishers reported fish aggregations in certain areas and at certain times, but while there was some agreement on aggregation timing, location and species, and while these are typical of spawning aggregations reported elsewhere, none of the interviewees mentioned spawning as the reason for aggregating.

Discussion
The results of the survey indicate that awareness of fish spawning aggregations among fishers in South Asia is low, with some notable exceptions. Possible reasons for this are manifold; we highlight the following:

- Few fishing methods involve fishers spending time in the water and directly observing reef fish. This is the case e.g. in Thailand (with the exception of indigenous sea gypsy communities), as well as in Aceh, the Lakshadweep Islands, Gulf of Mannar, and the Andaman Islands, where only some spear fishing is practiced, mainly in near shore and relatively shallow areas targeting octopi, lobster or reef fish. Spear fishing is more widely practiced in the Nicobar group of islands not covered by this survey. In Sri Lanka spear fishing using scuba and targeting groupers is common, but as the fishery is illegal fishers are reluctant to furbish information.
- Overfishing to a point where aggregations may have ceased, or at least been significantly reduced to densities hard to detect;
- Relatively low reliance on the demersal reef fish species that spawn in aggregations, as in Sri Lanka, where the Scombridae, Carangidae and Clupeidae make up a large part of fish landings (FAO 2006);
- Fish and fishing constitute supplements rather than the sole primary source of food and income in many communities particularly on the continental margin;
- Erosion of traditional knowledge, particularly in Lakshadweep, which in the 1960s and 70s saw a shift from reef fisheries to commercial tuna fishing as part

of a development programme. However, the reef fishery remains important for household consumption and many fishing methods are named after the target species, e.g. *Chammam fokkal*, "fishing for grouper" in Agatti (Hoon 2003).

The higher knowledge of reef fish spawning aggregations in the Maldives is a likely effect of the very high reliance on fisheries. While the pole and line tuna fishery has been the mainstay of the people over hundreds of years, coral reefs are used extensively for catching bait for the tuna fishery and reef fish have been a significant supplemental food source in many areas. The use of visually aided hook and line fishing for grouper means fishers spend long times in the water observing fish first hand. There was not a large difference in years of experience between fishers aware of spawning aggregations (49 interviewees, average 20 years of experience) and those not aware (seven interviewees, average 18 years of experience).

As the Maldives follows the Islamic lunar calendar, fishers are able to report lunar phase very accurately. Most respondents reported physical characteristics as the likely reason for spawning aggregations in a given location. Use of GPS among fishers is low; thus sites are defined by reef features and may in some instances be broad approximate areas, such as comparatively featureless reef slopes or sandy areas. Spawning aggregation depths estimated by fishers were dependent on visibility, and thus approximate. Fishers did not provide detailed information on tide and temperature. The tidal range in the area is modest, less than 1m, and temperatures very stable around 29-30, except in areas of localized upwelling.

Species

There is little information on spawning aggregations among the species identified in this study in Fishbase (Froese and Pauly 2008), while the SCRFA database includes records of confirmed spawning aggregations for most species reported here, including several records of *E. fuscoguttatus*, *E. polyphekadion, P. areolatus* from the Indo-Pacific; one record of *E. lanceolatus*, from Indonesia; a few records of *P. laevis*, *Cephalopholis miniata* and *Lutjanus bohar* from Papua New Guinea; some records of *Cheilinus undulatus* from Malaysia and Palau; and one record of *Caranx lugubris* reported from the Cayman Islands.

Three species for which potential spawning aggregations were recorded in this study are currently not included in the SCRFA database:

- *Plectropomus pessuliferus,* which is listed as a priority species for conservation due to its limited range, but not reported to form spawning aggregations (Domeier et al 2002).
- *Variola louti,* which may form spawning aggregations as indicated by observations from Papua New Guinea, with high densities including gravid females (Hamilton et al. 2005);
- *Caranx melampygus*, which is listed as forming spawning aggregations in Palau (Johannes 1981).

Aggregation spawning of *P. pessuliferus* is now confirmed in the Maldives. *C. melampygus* and *V. louti* were reported by only one respondent each in the Maldives, with unreliable reports of aggregation spawning of the latter in Aceh, and are thus not confirmed records.

Conclusions and Recommendations

While relatively limited in geographic scope and resolution, and while providing largely indicative results, this study is the first of its kind in the Bay of Bengal, and provides new information on indigenous knowledge of spawning behavior in high value reef fishes. Importantly, it also provides corroborated records of previously undocumented reef fish spawning aggregations in four atolls in the Maldives, including confirmation of aggregation spawning in one grouper previously not known to form spawning aggregations (*P. pessuliferus*).

The also provide useful information for fisheries management. One of the main threats to high value reef fish populations in the Bay of Bengal is the expanding market for chilled and live reef fish in East and Southeast Asia. A precautionary approach to settings export quotas as well as other restrictions could help prevent the overexploitation of fish spawning aggregations seen in Indonesia (Sadovy and Vincent 2002, Mous et al. 2000). A recent survey of grouper stocks in five atolls in the Maldives (MRC unpubl) confirms the decline in the fishery seen from export data (Sattar and Adam 2005), and fishers also reported reduced catches from spawning aggregations. Surveys in the Lakshadweep have shown that grouper diversity is relatively high and populations are recovering after what appears to be a reduction in abundance after the 1998 coral bleaching event (Arthur unpubl.). The *C. undulatus* density is representative of reefs with very low fishing pressure (Arthur unpubl., Sadovy et al. 2003). However, with continued demand, groupers, as well as other fish, are increasingly caught for cash income and export.

Further study is required to document in more detail spawning aggregations in the Bay of Bengal, at a higher resolution than in this initial survey. Such information is critical for fisheries as well as MPA

management planning. Sensitizing policy makers, managers and fishers regarding reef fish spawning aggregations, their conservation and management, remains a high priority for the region.

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Preliminary assessment of the socio-economic importance of export trade in coral reef resources on Fijian society

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Abstract. Rising demand for coral reef resources worldwide has led to an active international trade in reef organisms that are sourced primarily from tropical developing countries. Although these trades tend to be lucrative, it is unclear what economic benefit flows to the exporting country. We use Fiji as a case study to quantify the economic benefits of trade in coral reef resources to local communities. The live rock and aquarium fish trades provide the highest net income to collectors. However, the financial benefits of these trades are limited to a few villages. On the other hand, the bêche-de-mer fishery returns lower incomes to participants, but since the fishery has a large geographical coverage, more people are able to participate and obtain financial benefits from it. We find that the coral reef associated export trades generally provide a supplementary, rather than primary, source of income. This indicates that coastal communities should engage in diversified livelihoods. The unsustainable impacts of the export trade on the coral reef ecosystem reinforce the need for communities to be adaptable. As many coastal communities tend to fall back on subsistence fishing when a niche fishery declines, there is a need to better manage the extraction of coral reef resources in order to ensure their sustainability.

Key words: coral reef export trade, Fiji, socio-economics

Introduction

Coral reef resources such as fishes, invertebrates, and corals have long supported subsistence and artisanal fisheries that sustain the dietary and livelihood needs of coastal communities throughout the developing tropics (McManus 1997). It is widely acknowledged that growing populations and coastal development have increased pressure, and in many cases, led to unsustainable use of coral reef ecosystems (Pauly 1993). At the same time, the situation is exacerbated by the lucrative international coral reef trade, which is, in most cases, centered in wealthier, developed regions of the world. Demand for certain reef species for the live reef food fish, aquarium, and Chinese traditional medicine trades, for example, has led to intensive fishing and collection of certain reef species such as groupers, sea cucumbers, seahorses, corals, and butterfly fishes. The negative ecological impacts of these trades have been well documented (Sadovy et al. 2003; Lunn and Moreau 2004; Martin-Smith and Vincent 2006; Scales et al. 2006; Vincent et al. 2007).

What is not as commonly addressed is the extent to which trade in coral reef resources provides economic benefits to the exporting society. We address this question by using Fiji as a case study to examine the benefits the coral reef resource trade provides to local Fijian communities. Specifically, we look at the spatial extent of the coral export trade, and participants' economic dependence on these trades.

Fiji is an archipelago made up of 844 islands, cays, and islets, of which 106 are inhabited (Vuki et al. 2000). This group of islands is situated in the South Pacific between 15-23°S and 177-178°W, with a terrestrial area of 18,500 km^2 and a coastline of 1,130 km (CIA 2007). With approximately 1,000 coral reefs supporting about 200 species of corals (Rowlands et al. 2005) and around 1,200 marine fish species (Vuki et al. 2000), Fijian society has traditionally relied heavily on marine resources for subsistence and livelihoods. Marine resources have also become valuable export commodities, with the fisheries sector, inclusive of both coastal and offshore resources, contributing about 2.5% to Fiji's total GDP (ADB 2005).

The trade in marine aquarium organisms includes ornamental fish, invertebrates, coral, and live rock, and has been active in Fiji since the early 1970s (Yeeting 2007). Pacific island nations supply about 18% of the 3.4 million ornamental fishes traded on the international market, with Fiji alone contributing

5% (Wabnitz et al. 2003). Similarly, Pacific island nations supply 25% of internationally traded live corals, with Fiji supplying 4% (Wabnitz et al. 2003). In particular, demand for live rock from Fiji has grown steadily since the late 1990s (Lal and Cerelala 2005), due to its distinctive colour, and Fiji is now one of the major exporters of live rock. Overall, Fiji is ranked fourth after Indonesia, China and the Philippines in the export of marine aquarium products globally (Lal and Cerelala 2005). There are five companies involved in the trade of aquarium products in Fiji. These companies operate in 25 *qoliqoli* (traditional fishing grounds) that are located along the Coral Coast and islands off the Western Division (Lal and Cerelala 2005).

The bêche-de-mer (sea cucumber) trade has a long history in Fiji dating back to the 1800s. This sector exhibits a 'boom and bust' cycle, with the most recent boom taking place in the mid 1980s when production of dried bêche-de-mer in Fiji peaked at an estimated 1,000 tonnes in 1988 (Adams 1992). The collection of bêche-de-mer is largely carried out on a small scale in villages throughout Fiji. Bêche-de-mer is gleaned from shallow reefs by men, women, and children, or collected by men during dive fishing trips. Bêche-de-mer is sold either raw or dried to middlemen. Closer to urban areas, villagers may travel to sell their product, whereas in more remote regions, middlemen make collection trips to the village several times per month. Alternatively, company agents are posted at a village, and are responsible for collecting and bringing the product to market. Middlemen themselves may be agents for an export company, or may export the product directly to overseas markets in China and North America, among others.

The live reef food fishery (LRFF) was established in Fiji in 1998, targeting coral groupers (*Plectropomus* spp.) for markets in Hong Kong and Southeast Asia. It reached a maximum of eight companies before declining to one or two in recent years. Like the aquarium trade, LRFF companies have to negotiate a contract with *qoliqoli* owners. These companies employ fishers from local villages, and use mother vessels to collect fish from locations around Vanua Levu, which are then brought to Nadi for export (Yeeting 2001; Ovasisi 2006).

Coral reef resources provide a valuable source of export revenue to Fiji. From 2002 to 2004, exports of aquarium products, live reef food fish, bêche-de-mer, and trochus shells generated between USD 10.8 and USD 13.7 million in annual export revenue per year (Table 1). In comparison, domestic sales of reef-associated finfish and invertebrates in 2004 were estimated to have a gross value of USD 33.4 million (Starkhouse 2009).

Table 1. Export value (USD)[a] of coral reef resources 2002-2004, extracted from Fiji Department of Fisheries Annual Reports

Reef resource	2002	2003	2004
Aquarium products[b]	5,535,135	6,540,541	6,486,486
LRFF[c]	972,973	572,973	154,184
Bêche-de-mer	5,945,946	2,702,703	4,324,324
Trochus	1,259,459	978,378	810,811
TOTAL	**13,713,514**	**10,794,595**	**11,775,805**

[a] The exchange rate on 31 January 2009 was 1 USD = 1.85 FJD.
[b] Includes ornamental fish, live rock, live coral, live invertebrates, live clams, and unworked corals.
[c] Data for 2003 and 2004 obtained from Ovasisi (2006).

Materials and Methods

We interviewed 47 fishers and 10 middlemen and reef resource export companies in May and June 2008. Our interview team included two research officers from the Fiji Fisheries Department. Interview sites were chosen to provide a wide geographical coverage of Fiji as well as being inclusive of both artisanal and subsistence fishing communities. Fishers were chosen opportunistically – in most cases, we interviewed fishers who were assembled at a central location at the request of the village headman, from whom we had obtained permission to conduct interviews at the particular village. These included full and part time fishers, both men and women.

The interviews followed a semi-structured format where a prepared questionnaire was used to guide the interview, but respondents were free to elaborate on issues of interest. Our interviews focused on the economic aspects of the live reef food fish and bêche-de-mer fisheries, including information on fishers' catch, revenues and costs.

Fisher interviews were conducted in 13 villages and settlements in western and northern Viti Levu, the Yasawa islands, Bua Province in Vanua Levu, and Kadavu island south of Viti Levu. Interviews with middlemen and traders took place in major towns such as Suva, Lautoka, and Labasa. Published and grey literature, and statistics from the Fisheries Department Annual Reports and Secretariat of the Pacific Community were used to provide national level information on the aquarium, coral, and live rock trades.

Results

Spatial coverage and participation

The bêche-de-mer trade was the most geographically dispersed (Fig. 1), and involved the most number of participants as it is harvested by both adults and children in coastal villagers throughout Fiji. Fifty-one

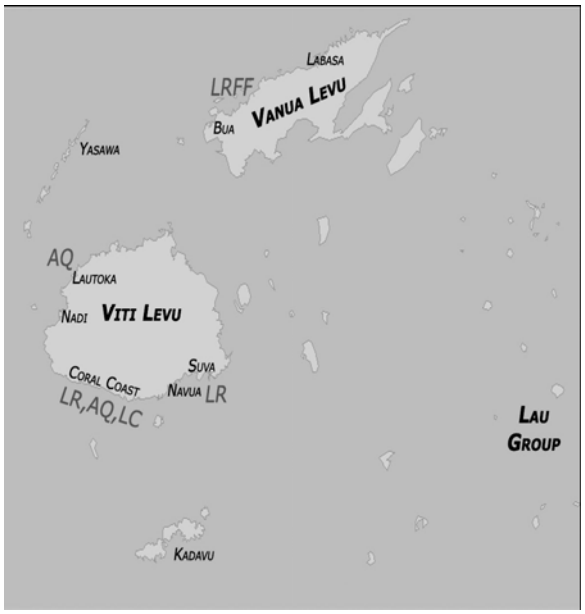

Figure 1: Map showing the spatial extent of the coral reef export trades. Note that there is no indicator for the bêche-de-mer trade because it occurs throughout Fiji. The other trades are:
LRFF=live reef food fish, AQ=aquarium fish, LR=live rock, and LC=coral.

percent of respondents collected bêche-de-mer. Of these, 71% also engaged in artisanal or subsistence fishing. In villages where bêche-de-mer was harvested, respondents often indicated that most women participated in collecting the invertebrates.

The live reef food fish trade (LRFF) is concentrated in one village on Tavea Island in Bua Province, Vanua Levu, where around 25 fishers participate in the fishery. The aquarium and live rock and coral trades are concentrated along the Coral Coast, and in 3 to 4 villages around Suva and Navua (Fig. 1). In a typical live rock operation along the Coral Coast, around 8 collectors were observed harvesting live rock. However, the number of harvesters varies, and can range from 2 or 3 to 8. Table 2 provides a summary of the spatial distribution of the coral reef trades in Fiji.

Table 2. Summary of spatial distribution of coral reef trades in Fiji.

TRADE	SPATIAL DISTRIBUTION	LOCATION
Bêche-de-mer	Widespread	Coastal villages throughout Viti Levu, Vanua Levu, Kadavu, Yasawa, Lau
Live reef food fish	Limited	1 village in Bua Province, Vanua Levu
Aquarium fish	Limited	Villages along Coral Coast & Lautoka
Live rock	Limited	3 villages along Coral Coast, & 3-4 villages around Suva and Navua*

* Source: Why and Tuwai (2005).

Economic benefits

Thirteen percent of respondents relied on a coral reef export trade for their main source of income, while for the rest it was a supplemental form of income (Table 3). The majority of those who depended on an export trade for their primary source of income were involved in the live reef food fish trade. Bêche-de-mer was the most common provider of supplemental income, and on average accounted for 29% of total fishing income.

Table 3. Level of dependence on coral reef export trades as a source of income

TRADE	INCOME SOURCE	
	Main	Supplemental
Live reef food fish	√	
Bêche-de-mer	√	√
Aquarium fish	√	
Live rock		√
Live coral		√

The net weekly income for collectors of reef resources for each coral reef trade is shown in Figure 2. Collection of aquarium fish generated the highest net weekly income (USD265), while harvesting live coral provided the least income (USD39). However, when compared to the 2002/2003 Fiji National Basic Needs Poverty Line income of USD97 (FJD178) household^{-1}week^{-1} (http://www.fiji.gov.fj/publish/page_5838.shtml), only the aquarium and live rock trades provided sufficient income to meet this baseline.

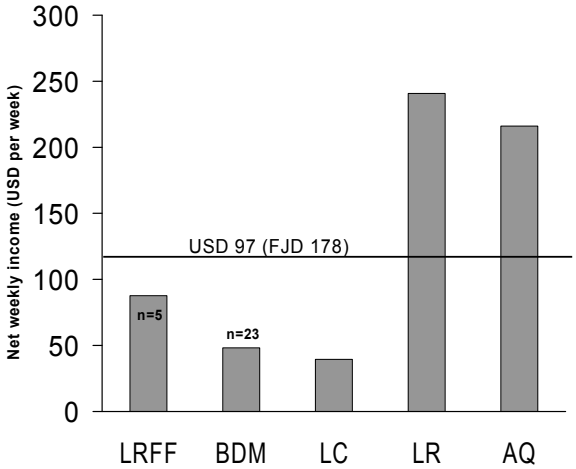

Figure 2: Net weekly income for participants in the coral reef export trades. The line shows the national basic needs poverty line income of USD97 household^{-1}week^{-1}. Results for LRFF (live reef food fish, mean ± standard deviation=USD87±26) and BDM (bêche-de-mer, USD48±11) are derived from fisher interviews, with sample sizes (n) indicated. Results for AQ (aquarium fish, USD 216) are based on an interview with an aquarium fish exporter in Fiji. Data for LC= live coral (USD40) and LR=live rock (USD241) are from Lal and Cerelala (2005).

Discussion

We aimed to quantify the economic benefits of the export trade in coral reef resources to Fijian communities. Our findings indicate that on the whole, the coral reef resources export trade provides economic benefits to coastal villagers in the 8 study sites. We recognise that our small sample size may limit the ability to generalize this study to Fiji as a whole. However, the consistent responses we obtained from interviewees across a wide geographical coverage indicate that our findings are likely applicable throughout Fiji. In any case, this study is useful for providing a preliminary overview and identifying broad patterns of how the trade in coral reef resources affects communities in different parts of Fiji.

The bêche-de-mer trade provides the most widespread benefits, as it occurs near shore and in most cases requires no specialized gear[1], such that almost all members of society are able to participate in it. In fact, Fijians have been involved in the bêche-de-mer trade for over a century to supply the international demand for these invertebrates (Adams 1992).

In contrast, the live reef food fish trade is limited to around 25 participants in one village, and although it provides the primary source of income for these participants, the trade only makes a limited contribution to Fijian communities on the societal level. Similarly, the economic benefits of the aquarium fish and live rock trades are limited to villagers in a specific region of Fiji. The relatively low level of participation in the aquarium and live reef food fish trades can be attributed to the need for special skills. Aquarium fish collectors use SCUBA equipment to collect the target fish species. The equipment, as well as SCUBA training, is provided by the aquarium fish company. Similarly, live reef food fish fishers were trained on how to handle live fish, as fishing for live fish is not a traditional Fijian fishing technique. On the other hand, the collection of live rock and coral is constrained to distinct villages along the Coral Coast due to the presence of fringing reefs along this area.

Our preliminary findings indicate that overall, coral reef export trade is a supplemental, rather than primary, source of income for coastal villagers. In fact, as shown in Figure 2, in most cases, depending on one export trade alone for income is not sufficient to meet minimal household expenditure needs. For example, we observed that women tended to use proceeds from selling bêche-de-mer to pay for day to day household requirements, while big ticket items tended to be purchased and paid off from the domestic sale of fresh fish. This indicates that for participants in the coral reef export trades, it is critical to pursue a diversified livelihood strategy, and, therefore, that the condition of reef resources in coastal areas is important for all fishing community members. Our findings, as well as those of other surveys (e.g., see van Beukering et al. 2007; Veitayaki et al. undated), show that many villagers do in fact adopt a diversified livelihood strategy. For instance, the vast majority (71%) of respondents who collected bêche-de-mer also engaged in other fisheries. Moreover, in addition to participating in the reef export trades, many respondents also engaged in multiple subsistence activities such as planting crops and handicraft making, or finding temporary paid employment.

Engaging in a variety of income-earning activities is consistent with socio-economic studies in other parts of Fiji (Veitayaki et al. undated; Turner et al. 2007), and is an important strategy for ensuring sustainable livelihoods (Allison and Ellis 2001). Having the ability to adapt to another livelihood is particularly important given the ecological concerns connected with the coral reef export trades (e.g., Kolm and Berghund 2003; Sadovy et al. 2003; Rowlands et al. 2005; Why and Tuwai 2005).

The sustainability of bêche-de-mer exploitation in Fiji has been a concern since the 1990s (Adams 1992), as holothurians tend to be susceptible to overfishing (Uthicke and Conand 2005). In at least two villages we visited, villagers no longer collected bêche-de-mer as the invertebrates could not be found anymore. Similarly, adverse ecological impacts have been documented in connection with the collection of live rock and aquarium fish in Fiji (Sykes et al. 2003; Why and Tuwai 2005). Likewise, the live reef food fish trade has led to the collapse of these targeted fisheries in parts of Southeast Asia (Sadovy et al. 2003; Scales et al. 2006). There are indications that the live reef food fish fishery based in Bua Province is experiencing a decline too, as interviewed fishers claim that it is increasingly difficult to catch the targeted grouper species.

When an export market fails, for example through local species extirpation or the departure of a buyer, fishing provides a safety net for villagers. This was the case when a live reef food fish company departed from a village in Vanua Levu, and when the bêche-de-mer fishery collapsed in other villages. As such, protecting the health of marine resources in Fiji is crucial not only to maintain economic benefits from the export trade in coral reef resources, but also, to maintain the survival of coastal communities when that same trade fails.

[1] In some villages, fishers may use SCUBA gear to collect bêche-de-mer species that occur at greater depth.

In conclusion, the coral reef export trade in Fiji can be considered economically beneficial to coastal communities. However, for many participants these trades are mainly a source of supplemental, rather than primary, income. The bêche-de-mer trade provides the most widespread benefits to communities throughout Fiji in terms of being accessible to the greatest number of participants. The live rock and aquarium fish trades provide the highest net income to collectors, but the benefits of these trades are limited to a few villages. The ecological impact of coral reef export trades, together with the fact that it cannot provide a primary source of income, underlines the importance of pursuing a diversified livelihood. At the same time, management policies should be strengthened to ensure the sustainability of inshore fisheries resources, as villagers continue to rely on these marine resources as their safety net when certain coral reef export trades fail.

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Improving management of coral reefs fisheries in data limited situations: Experiences from the ParFish methodology

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Abstract. Coral reef fisheries are complex systems. This is due primarily to the multi-species, multi-gear and high labor mobility that often occurs within the fisheries sector. Since many small-scale fisheries operate in remote areas and/ or in developing coastal states, the quality and quantity of data necessary to undertake basic fisheries management, including robust stock assessments, are often lacking. It is becoming more widely acknowledged that local stakeholder participation is an essential pre-requisite to improve overall management of the resource, but this has rarely engaged them directly within a stock assessment framework to provide information on the status of the resource. This study presents a summary and comparison of three pilot studies undertaken in Turks and Caicos Islands, Zanzibar and Puerto Rico. Through the development of a participatory fish stock assessment (ParFish) methodology, fishers are engaged at the beginning of an ongoing adaptive management process to contribute their valuable knowledge about the fishery. Using a Bayesian statistical model, information obtained from fishers help inform prior statistical distributions that are used to estimate parameters in the stock assessment model. The results have shown that this approach can be used successfully to establish preliminary results of stock status in data limited situations.

Key words: Reef fisheries, Data limited, Stakeholder participation, Stock assessment, ParFish

Introduction

The future health and status of coral reefs are threatened from many sources, including environmental and anthropogenic impacts such as climate change, pollution and fisheries (Carpenter et al. 2008). Coral reef fisheries provide a valuable source of income and source of protein for many coastal communities, although the status of reef fisheries has been exacerbated by poor management (Munro 1996).

One important pre-requisite for effective fisheries management is information on the status of the resource. However, in many regions of the world, fisheries data are often lacking due to limited funding and capacity within fisheries management agencies. A lack of quantitative fisheries information has prohibited the use of traditional stock assessment models to assess the status of the stocks, without which sustainable catch control limits cannot be determined. Moreover, without effective monitoring, control and surveillance, illegal, unreported and unregulated fishing is likely to undermine existing management measures.

Fisheries management has now recognized the importance of stakeholder participation, and the potential benefits of co-management initiatives are well documented (Wilson et al. 2004). Until recently, however, fishers and key stakeholders have not been directly involved in the assessment of the stock.

This paper describes the results from three pilot studies that have been used to test a rapid assessment technique that uses fishers' knowledge and information to parameterize traditional stock assessment models without need of a long time-series of data. The results of the Participatory Fish Stock Assessment (ParFish) methodology (Medley 2006) are described in an attempt to determine if it can be used successfully in the field.

Material and Methods

The ParFish methodology was developed under the Fisheries Management Science Programme (FMSP) (www.fmsp.co.uk) funded by the UK Department for International Development (DFID). It is an adaptive process that includes six key stages to (i) understand the context of the fishery (ii) agree objectives of the study with stakeholders (iii) undertake the ParFish

stock assessment (iv) give feedback of the results and initiate management planning (v) assist with the implementation of the management plans, and (vi) evaluate the ParFish process.

The ParFish approach is supported by two main components: a toolkit that provides guidance on the process outlined above and the ParFish software. The software enables the analysis of the data, based on Bayesian statistics and decision analysis. Quantitative data used to develop statistical probability distributions (or priors) of parameters used in the stock assessment are obtained from interviews with fishers. In addition, preference interviews are used to score individual fishers' utility (i.e. risk seeking or risk averse) to selecting different management options based on changes in fishing effort (cost) and expected catch (benefit). A full description of the ParFish methodology and toolkit can be found in Walmsley et al. (2005).

Development of ParFish is ongoing, and this study presents a summary of the results obtained from pilot studies occurring within the Caribbean region (Turks and Caicos Islands and Puerto Rico) and Africa (Zanzibar Island, United Rep. Tanzania).

Turks and Caicos Islands

A pilot study funded by DFID was conducted to assess the status of the queen conch (*Strombus gigas*) on the islands of Provenciales and South Caicos (Fig. 1). This fishery provided a useful test for the value of the fisher interviews since a long time series of catch and effort data is available for comparison.

The fishery is targeted by small day boats with fishers who free dive to 10m depth to collect conch that is shelled at sea. The meat is landed and recorded at processing plants. These data, available since 1974, are used to record catch and effort information and have subsequently been used to estimate stock size from a logistic biomass model (Medley and Ninnes 1999). The data collected from processing plants are used by the government to set catch quotas for landings and exports (Taylor and Medley 2003a).

With the assistance of the Department of Environment and Coastal Resources (DECR), a total of 46 stock assessment and 38 fisher preference interviews were conducted between 3rd and 21st July, 2003.

In addition to the comparison between the stock assessment and the ParFish results, a simple retrospective analysis was performed to see if the interviews alone might have improved the management of the fishery, had that information been used to set effort control back in 1974.

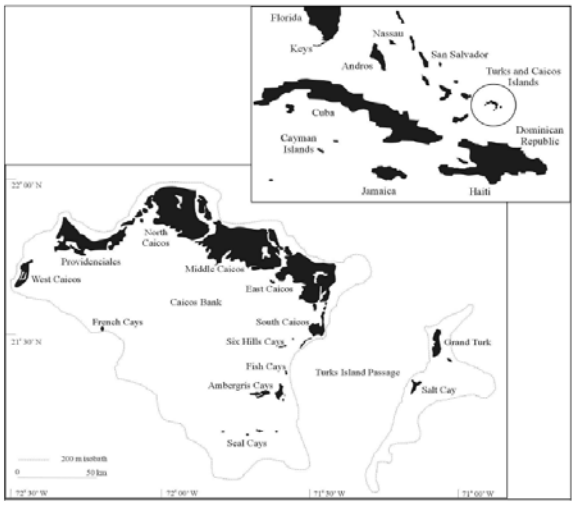

Figure 1: Location of ParFish assessment in Provenciales and South Caicos within Turks and Caicos Islands during ParFish assessment.

Zanzibar

A pilot study funded by DFID was conducted to assess the status of a multi-species fishery on both the offshore patch reefs (Dimbani) and inshore fringing reefs (Mkunguni and Mtende) in the Kizimkazi region of southern Zanzibar (Fig. 2). The field sites also fall within the boundaries of the Menai Bay Conservation Area which was listed as a multiple-use management area in 1997 (ref here). To date, no restrictions are placed on the minimum size of capture or catch limits.

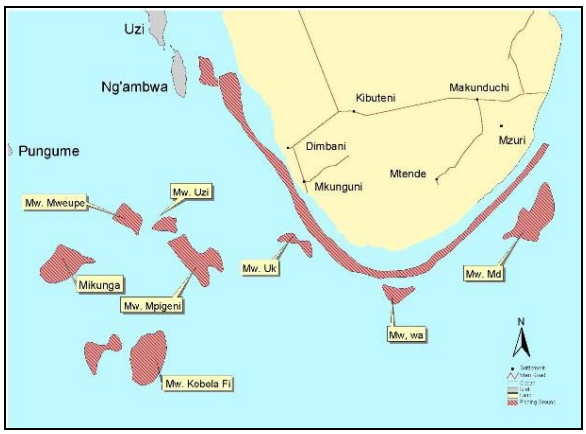

Figure 2: Location of ParFish assessment in Kizimkazi, southern Zanzibar Island during ParFish assessment.

The fishery provided a further opportunity to develop the ParFish interview technique and develop the participatory process but also included a range of auxiliary information from research experiments. This included fishing experiments, underwater visual census (UVC) and mark and recapture of fish within the experimental area (Taylor and Medley 2003b).

With the collaboration of the Institute of Marine Science, University of Dar es Salaam, a total of 92

stock assessment and 67 preference interviews were completed between April and June, 2003.

Fishing Experiments

Depletion techniques reduce the population size through fishing to provide parameters necessary to estimate stock size (Hilborn and Walters, 1992). An important assumption of depletion methods is that no immigration or emigration occurs in the population during the experiment, and thus would preferably be conducted over a short time period. In Dimbani (offshore reef), the fishing experiment was conducted over an area of 600 x 500m during an eight day period with approximately 55 fishers while in Mkunguni and Mtende (inshore reef), this was conducted within an area of 800 x 100m with an average of 43 fishers during a nine day period. Log books were provided to all fishermen to record fishing duration and catch by species.

Underwater Visual Census (UVC)

A UVC, using similar methods to Gaudian et al. (1995), was conducted at Mkunguni and Mtende (inshore reef sites). Six permanent monitoring stations were established in the study area: two within the fishing area for the depletion experiment and two on either side of the experiment area. Each transect (200 x 10m belt) was conducted by two pairs of divers, each recording the target species within the transect boundaries. These sites were monitored at random (as far as possible) before, during, immediately after, and one month after the fishing experiment.

Mark and Recapture Studies

The use of mark and recapture techniques in the ParFish experimental design was investigated at Mkunguni and Mtende (inshore reef sites). Following the demarcation of the fishing experiment, an intensive tagging program was conducted during eight days within the same area. During the tagging phase 566 fish from 35 different species were marked and released within the boundaries of the fishing area. The depletion experiment was then used to recapture the tagged fish, in addition to recording tagged fish during the UVC.

Puerto Rico

A pilot study funded by the NOAA Cooperative Research Program was conducted to assess the status of the two most important deepwater snapper: queen snapper (*Etelis oculatus*) and silk snapper (*Lutjanus vivanus*) in western Puerto Rico. The study enabled the ParFish software to be further developed to include a yield-per-recruit analysis in addition to the existing logistic production model.

Deepwater snapper are targeted by a small-scale hand line fishery using electric reels and multiple baited hooks. Approximately 30 to 40 full-time commercial fishers operate from two main fishing centers, Rincón and Cabo Rojo. Standardized catch and effort data are available through SEAMAP surveys (Rosario Jimenez 1989), although these have been shown to be of limited use for the assessment of the snapper complex (Ault and Rothchild 1991). To date, management of the fishery employs a closed season for silk snapper and minimum size limits for both species. There are no catch limits placed on either species.

With the assistance of the Department of Natural Resources and the Environment, a total of 31 stock assessment and 29 preference interviews were conducted between March and June 2008.

Results

Turks and Caicos Islands

The results of the ParFish stock assessment are associated with high uncertainty in the status of the conch stock, although overall there is a greater tendency to indicate the stock is overfished. The scientific data from the catch and effort time series supports the fishers' view that the fishery is in an overfished state. When interview and scientific data are combined the posterior opinion is that the fishery is overfished (Fig. 3).

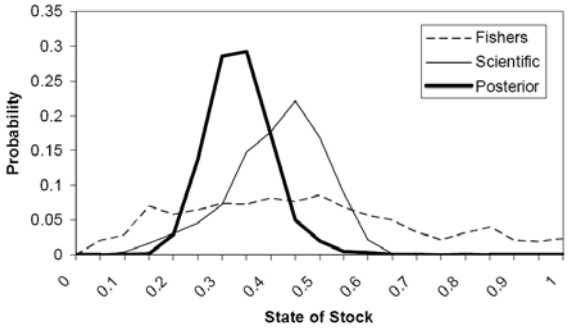

Figure 3: State of stock (current biomass / initial biomass) estimated from ParFish interviews, scientific catch and effort time series data and combined analysis (posterior distribution).

Similarly, the ParFish assessment showed high uncertainty in the level of fishing effort although overall there was a higher probability that overfishing was occurring, or at the very least fishing at the maximum (status greater than 1). Again, the scientific data supports the fishers' view that the stock is overfished. When interview and scientific data are combined, the posterior opinion shows that the stock is either currently overfished or very close to being in an overfished state (Fig. 4).

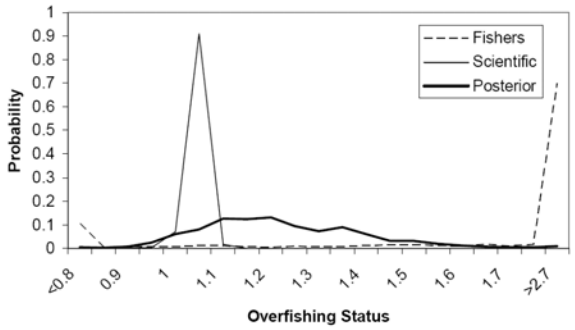

Figure 4: Overfishing status of stock (current fishing mortality / fishing mortality at MSY) estimated from ParFish interviews, scientific catch and effort time series and combined analysis (posterior distribution).

Since the fishery is managed on a catch quota system, the results from the ParFish stock assessment and preference interviews were used as part of a retrospective analysis (Fig. 5) to evaluate the impacts if an appropriate catch level had been applied back in 1974.

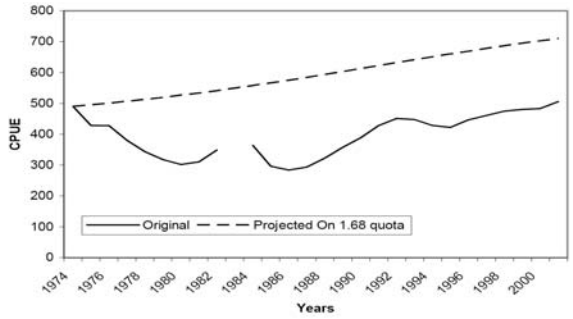

Figure 5: Expected catch per boat day (CPUE) from the fitted logistic model (original) and the projected CPUE from the annual catch quota (1.68 m pound) estimated from ParFish interviews.

Assuming the logistical model can represent the dynamics of the fishery, the results show that the total catches over the period 1975-2002 were very similar (approx. 46 million pounds; not shown). There was, however, a marked increase in the catch rate. This suggests that far less effort (and costs) would have had to be exerted than is now applied (from 3,300 boat days down to 2,500 boat days).

Zanzibar

The results of the ParFish assessment indicate that the current states of both the offshore and fringing reef multi-species fisheries are unknown, although the balance of probabilities suggests that the offshore fishery is overexploited. The results from the interviews suggest that too little is known about the initial state of the stock, which has been exploited for generations and is likely to provide unreliable estimates of unexploited biomass. In addition, there is no evidence to indicate overfishing is occurring in either fishery, although further increases in fishing effort should be capped until more information on the state of the stock can be determined.

The results of the fishing experiments showed that fishers were able to deplete the population on a local scale for both offshore and fringing reef populations sufficiently to estimate initial biomass that could be used in the ParFish assessment (Fig. 6).

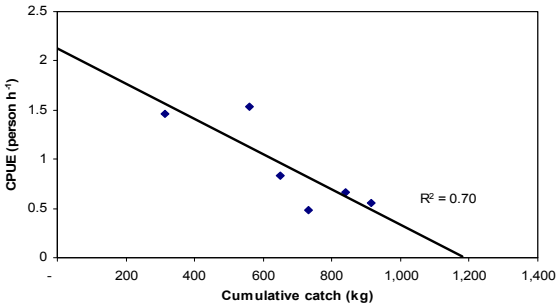

Figure 6: CPUE against cumulative catch to show depletion of population on a local scale for fringing reef fishery. Data represents days 4 -9 of the experiment.

The results of the UVC demonstrated that the abundance of the selected target species was reduced by the fishing experiment (Fig. 7). The increase in number of fish reported one month after the experiment indicates the population may experience immigration on a small scale.

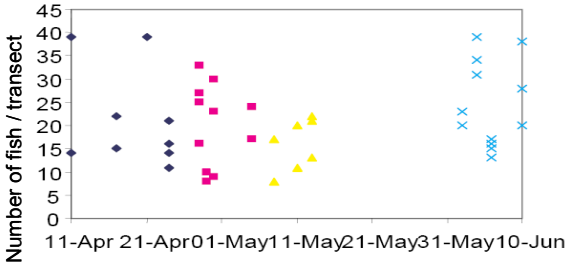

Figure 7: Density of fish observed at monitoring station on fringing reef, before (diamonds), during (squares), immediately after (triangles) and 1 month after (crosses) the fishing experiment.

During the fishing experiment, 56 tagged fish were recovered and provided a fishery-independent estimate of population size for the fishery. The mark and recapture data could also be used for estimating the population sizes of individual species for inclusion in multi-species assessments.

Puerto Rico

The assessment is still in process and the results show a high uncertainty associated with the parameters but that the probability of the current level of biomass for queen snapper being below the MSY level is slightly higher than 50% (i.e. possible overfished). In addition, the current yield from the analysis suggests that overfishing is not occurring.

Discussion

The results of the pilot studies have demonstrated that the ParFish methodology can be used to collect quantitative information from small-scale fisheries sufficient to parameterize traditional stock assessment models such as the logistical production and yield-per-recruit models using a Bayesian statistical framework. The methodology provides a rapid assessment of the stock, which does not require a long time-series of catch and effort data.

The robustness of the assessment results can depend on the quality and quantity of interview data obtained. It is therefore important to develop a good rapport with fishers and key stakeholders at the beginning of the study. This may require considerable outreach, but can help manage expectations and indicate the likely benefits of the results. Furthermore, it can lead to more effective involvement of fishers in the management process and development of regulations.

In comparison with more formal stock assessments, the initial ParFish results can exhibit high levels of uncertainty. This is to be expected however, given the diverse array of opinions and level of fisher experience. Options to help reduce the overall level of uncertainty in the results may include (i) ensuring that all fishers fully understand the questions being asked (ii) re-interviewing fishers with different questions that lead to the same answer, and (iii) asking very specific questions about a fixed attribute (e.g., closed area/ season etc.) that can be compared among fishers to measure sampling variability.

ParFish should not be viewed as a single one-off assessment, but part of an ongoing participatory adaptive learning and management process. To further help reduce the level of uncertainty in the results, information gathered from fisher preference interviews can be used to establish a consensus of opinion with management agencies to develop alternative levels of fishing effort. For example, fishers in Zanzibar have recognized that a reduction in fishing effort can be implemented by better enforcement of existing regulations to prevent illegal fishers from entering the fishery. Within a ParFish full assessment, these would become re-evaluated with new research and data collection programs to ensure catch rates can be improved by lowering fishing effort.

In conclusion, the ParFish methodology may be used where little or no previous data are available to conduct more formal stock assessments. It can be a useful tool in helping to establish initial catch limits and develop management options in collaboration with the fishers. A full ParFish assessment would include ongoing adaptive learning and management to further improve catch rates within the fishery and reduce the level of uncertainty in the results.

Acknowledgement

ParFish was originally developed under the Fisheries Science Management Programme funded by the UK Department for International Development (DFID) and further developed under the NOAA-funded Cooperative Research Programe. A number of people and institutions were instrumental in its development, in particular, Dr Narriman Jiddawi, Dr Omar Amir and Saleh Yahya, Institute of Marine Sciences, Zanzibar; Charlotte Tindall, MRAG; Oliver Taylor, Five Oceans Environmental Services LLC; Nancie Cummings, SEFSC, Miami; Eugenio Piñeiro, CFMC. We would also like to thank all the fishers who were involved in the ParFish interviews.

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Short term response of coral reef fish communities under customary management in New Ireland, Papua New Guinea

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Abstract. Papua New Guinea supports some of the most healthy and biologically diverse coral reefs. Coastal communities have customary tenure over fringing reefs and inshore fishing resources. Closure of an area of reef to harvesting (*tambu*) has historically been employed as a form of customary management. This study investigates the short-term response to this kind of management of moderately exploited coral reef fish communities. Fish biomass and abundance were surveyed by underwater visual census at three managed sites immediately before closure and at three nearby fished control sites at the 4m and 7m depth contours. This survey was repeated one year after closure. Our results display a significant increase in overall biomass within managed areas compared to control sites. We recorded significant increases in biomass and density of piscivorous fish at shallow transects in the managed areas, whilst piscivore biomass significantly decreased within shallow transects at control sites. Overall density of the fish communities remained largely unchanged. Serranids and Lutjanids showed the greatest change in biomass and density in managed areas. These short term positive results can be used to encourage communities to restore customary resource management.

Key words: Customary management, reef fish, Papua New Guinea, marine protected areas, no-take zones

Introduction

It is now well established on a variety of scales and in different regions of the world that marine protected areas (MPAs), in particular well enforced no take zones (NTZs) are effective at promoting recovery of reef fish populations (McClanahan 1994, McClanahan and Kaunda–Arara 1996, Halpern and Warner 2002, Russ et al. 2005). There remains some debate over the mechanisms and speed of recovery of a fish community from high fishing pressure (Halpern and Warner 2002, Russ et al. 2005). Often there is a large initial response to protection from harvesting (Halpern and Warner 2002), however studies in Kenya and the Philippines have indicated that the trajectory of recovery slows and full recovery from fishing may require more than a decade to occur (Russ and Alcala 2003, McClanahan and Graham 2005).

In Melanesia systems of customary marine tenure, heavy reliance on marine resources and heterogeneous perceptions of the status of marine resources (Cinner et al. 2005, Turner et al. 2007) have resulted in the failure of large-scale MPAs through top-down governmental processes as a conservation tool (McClanahan et al. 2006). In Papua New Guinea (PNG) historical and cultural factors have combined to form a regime of customary marine tenure that operates at the family, clan and village levels. This tenure and rights over the fishing resources within the tenure is legally recognised in PNG and provides a potentially useful tool in establishing small scale NTZs to protect reefs, provide refugia for fishery species and promote community involvement in conservation.

This study is part of ongoing research and monitoring of customary no harvesting (*tambu*) areas within the tenure of villages that are dependent on fishing. The work has been conducted by the Wildlife Conservation Society - Papua New Guinea Marine Program (WCS) in New Ireland Province in the Bismarck Archipelago (Fig 1.) Previous studies in the region have focused on spatial comparisons of *tambu* areas and other areas under management or open to fishing; this study is unique in the area, providing a temporal investigation into the effects of small-scale community managed areas in PNG.

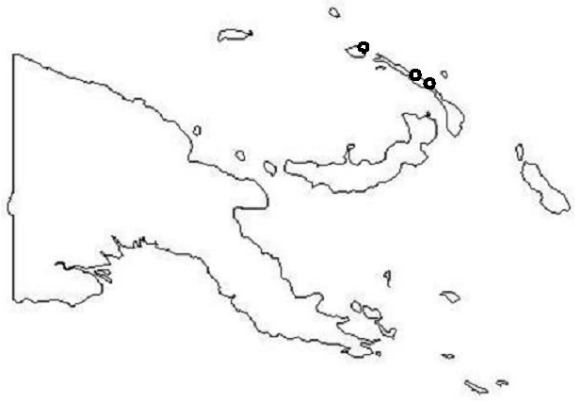

Figure 1. The three survey locations in New Ireland Province, PNG

Methods

Three subsistence fishing communities were identified within New Ireland Province, PNG by WCS in 2006 (Fig. 1). One (Ungakum) is situated in the Tsoi Islands approximately 35km Northwest of the provincial capital Kavieng, and the other two (Lasigi and Silom) are situated between 160 and 200km Southeast of Kavieng in central New Ireland. Criteria for selection of communities were that they were receptive to the reintroduction of customary fisheries management strategies, able to drive the process in partnership with WCS and were close to comparable communities that could be used as control areas. Each area (*tambus* and controls) stretches for between one and one and a half kilometres of fringing reef, and from the shoreline to the bottom of the reef slope or the 30m depth contour.

Through underwater visual census (UVC) the abundances of all non-cryptic, diurnal species of reef fishes >3cm total length (TL) were recorded along two 50m transects at the 4m and 7m depth contours at six sites within each area. At each transect two passes were made, the first recording large fishes (>10cm TL) 2.5m either side of the transect tape, the second recording small fishes (≤10cm TL) 1m either side of the transect tape. All individuals were placed within the appropriate 5cm size class (i.e. 3-5cm, 6-10cm, 11-15cm etc.). This survey method was employed across three customary managed no-take reserves, initially during September/October 2006 (prior to closure to harvesting) followed by a repeat survey twelve months later. Each managed area of reef was paired with a control (fished) area selected on the grounds of being ecologically and geomorphologically similar and situated between 1 and 5km from the managed site. These control sites were surveyed immediately after each adjacent managed area in both 2006 and 2007.

Biomass estimates were obtained using length-weight conversion factors obtained from Fishbase (Froese and Pauly 2006). Length data used in biomass calculations used the midpoint of size classes. Before any parametric statistical test, data met all assumptions for normality.

Results

After 12 months of closure our results showed six major trends:

1. After one year of closure at the managed areas we recorded a highly significant increase in overall mean fish biomass (one way ANOVA P=0.001) (Fig. 2a) compared with no significant change at the fished control areas (P= 0.537).
2. Overall fish community density showed no significant change in managed areas or corresponding control areas over the same time period (Fig. 2 b).

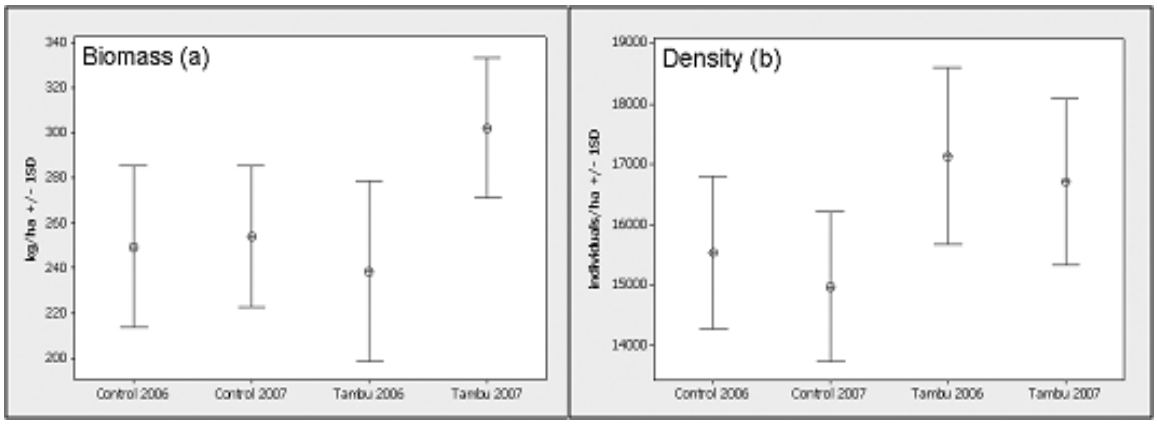

Figure 2. Overall coral reef fish community biomass (kg/ha ±1SD) (a) and density (individual fish/ha ±1SD) (b) at customary managed areas (*tambu*) (2006 and 2007), compared with the three control areas (2006 and 2007).

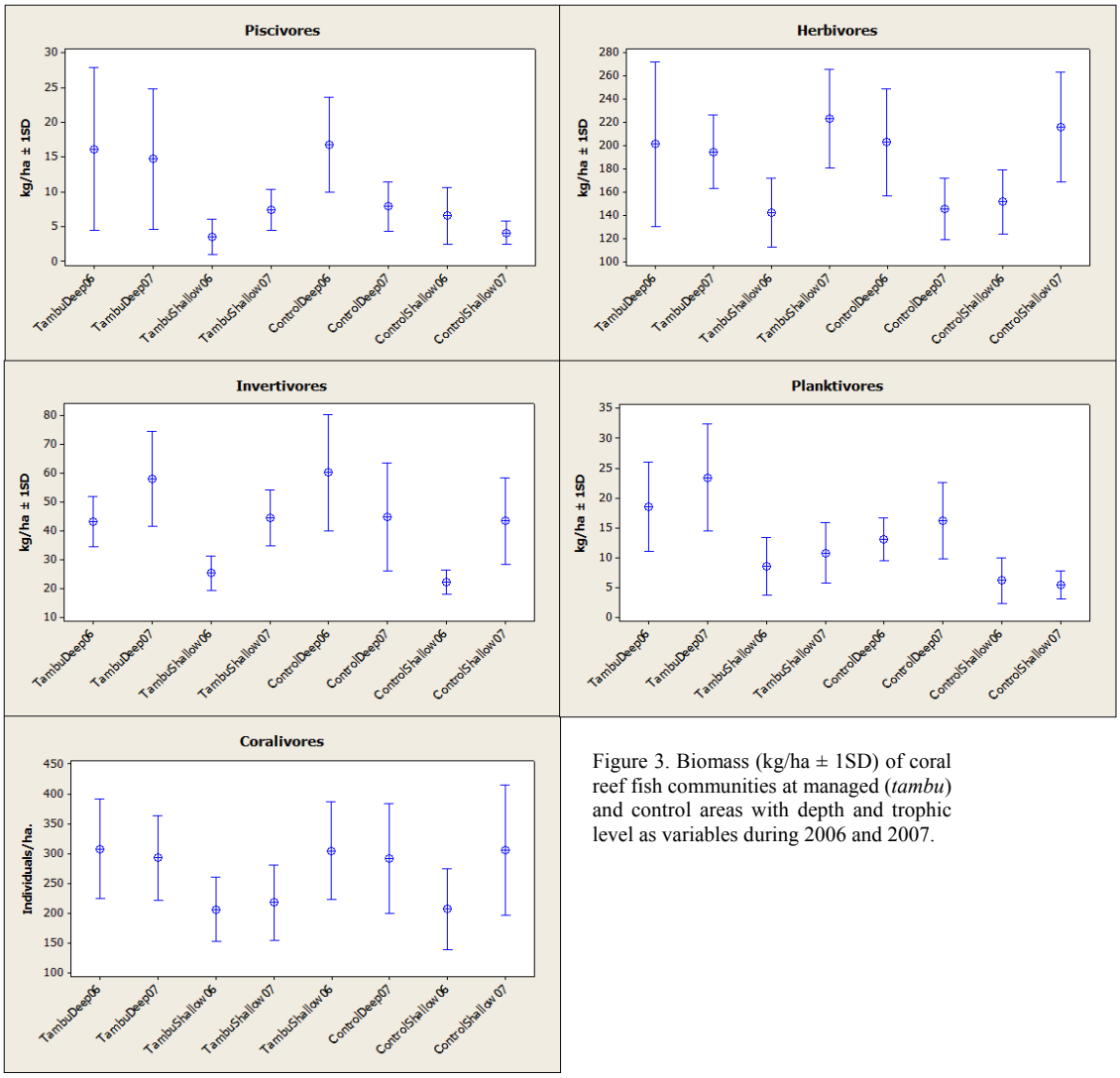

Figure 3. Biomass (kg/ha ± 1SD) of coral reef fish communities at managed (*tambu*) and control areas with depth and trophic level as variables during 2006 and 2007.

Table 1. *P* values (one way ANOVA) of coral reef fish community biomass (kg/ha) displayed in Figure 3 (2006 vs 2007), at trophic level for shallow and deep sites. All assumptions of normality were met for data.

	Tambu	***Control***	***Tambu***	***Control***	***Tambu***	***Control***	***Tambu***	***Control***	***Tambu***	***Control***
	Piscivores		***Herbivores***		***Invertivores***		***Planktivores***		***Coralivores***	
Deep	0.693	0.284	0.220	0.026	0.118	0.061	0.993	0.871	0.090	0.952
Shallow	<0.000	0.228	0.003	0.019	<0.000	0.001	0.414	0.887	0.001	0.007

3 The greatest change in biomass occurred within piscivores at shallow sites. We recorded a highly significant mean increase of 3.83 kg/ha at the managed shallow sites (P<0.000), compared to a non-significant mean decrease of 2.43 kg/ha recorded at the shallow control sites (Fig 3, Table 1). Other changes in biomass were evident across the trophic structure of the fish community, including significant increases in invertivore biomass at both *tambu* and control areas, however none displayed such a large variation between the managed areas and control areas (Fig. 3).

4 There was very little change in the density of the fish population at the trophic level, with the exception of a highly significant mean increase of 115.5 individuals/ha of piscivores (P=<0.000) (Fig. 4, Table 2) at the shallow managed sites.

5 There was a 91.33% dissimilarity between the piscivore community biomass structure at the shallow managed sites between 2006 and 2007 (SIMPER; Table 3).

Table 2. Resulting *P* values (one way ANOVA) of coral reef fish community density (individuals/ha) displayed in Figure 4 (2006 vs 2007), at trophic level for shallow and deep sites. All assumptions of normality were met for data.

	Tambu	*Control*	*Tambu*	*Control*	*Tambu*	*Control*	*Tambu*	*Control*	*Tambu*	*Control*
	Piscivores		*Herbivores*		*Invertivores*		*Planktivores*		*Coralivores*	
Deep	0.061	0.334	0.608	0.179	0.547	0.125	0.532	0.433	0.774	0.834
Shallow	<0.000	0.101	0.091	0.350	0.446	0.123	0.620	0.459	0.777	0.124

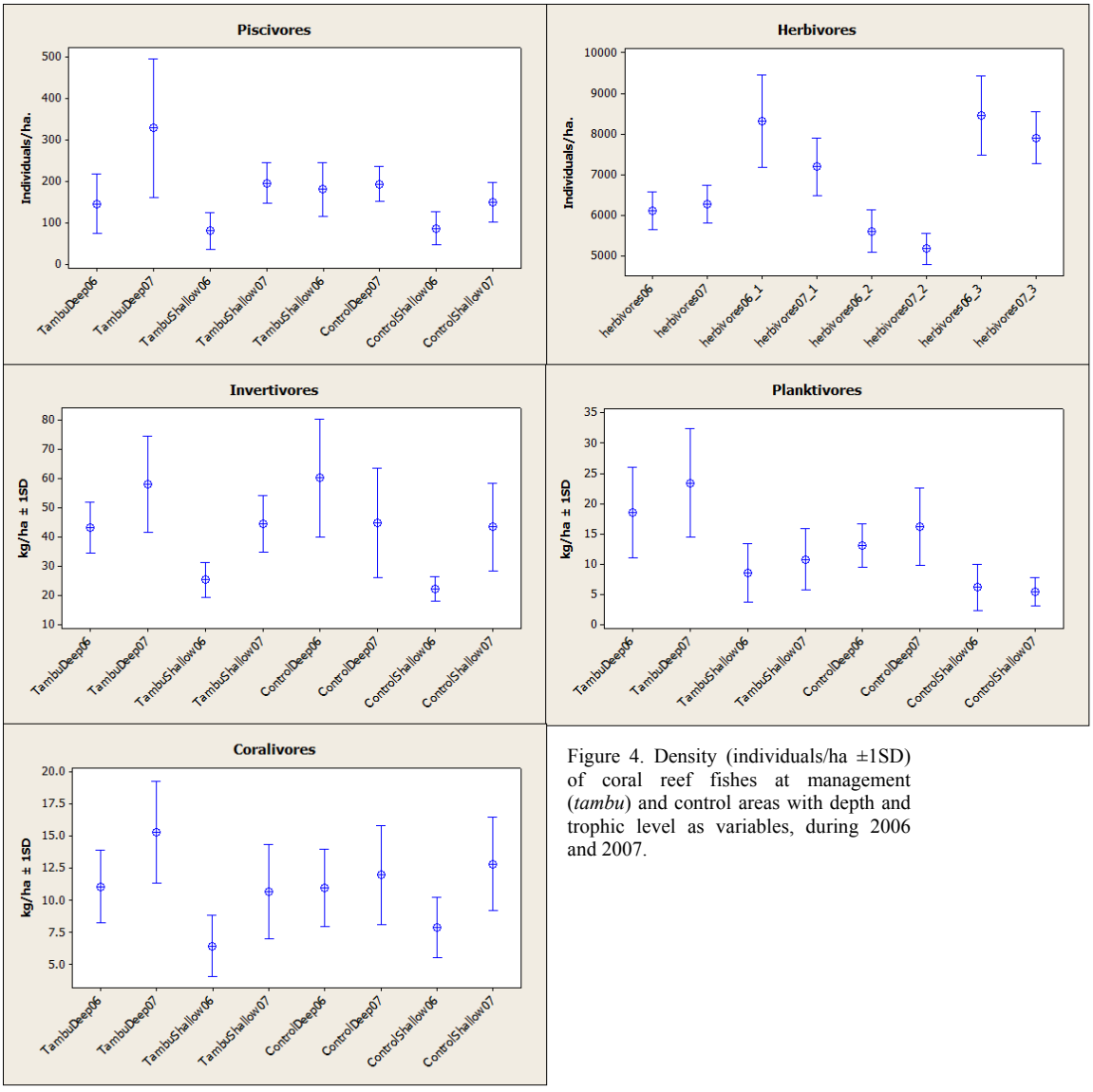

Figure 4. Density (individuals/ha ±1SD) of coral reef fishes at management (*tambu*) and control areas with depth and trophic level as variables, during 2006 and 2007.

6 Serranidae and Lutjanidae contributed 45.01% and 41.46% respectively of the dissimilarity in biomass, with very similar results for fish density (SIMPER; Table 3).

Discussion

Our results support the claims that the biological responses as a result of implementing NTZs appear to develop quickly in some cases. Indeed, Halpern and Warner (2002) state this is often the case and in their review show that NTZs can support higher than average values of density, biomass, average organism size and diversity inside reserves (relative to controls) within a short (1–3yr) time period. Our results are comparable with results from the Great Barrier Reef collated by Evans *et al.* (2006). This study showed

Table 3. SIMPER output of data for biomass of piscivorous families at the shallow managed sites showing change in the percent make up of the community structure between closure (2006) and after one year of closure (2007).

Average dissimilarity = 91.33						
	Group 2006	**Group 2007**				
Species	*Av. Biomass*	*Av. Biomass*	*Av. Diss*	*Diss./SD*	*Contrib.%*	*Cum.%*
Serranidae	0.31	0.4	41.1	0.93	45.01	45.01
Lutjanidae	0.18	0.47	37.87	0.88	41.46	86.47
Lethrinidae	0	0.12	6.76	0.28	7.4	93.87
Haemulidae	0	0.1	5.6	0.27	6.13	100

that after just 21 months of closure to fishing, the density and biomass of coral trout (*Plectropomus* spp., family Serranidae), and the lutjanid *Lutjanus carponotatus* had increased by up to a factor of 1.7 in areas that had been closed to fishing, while density and biomass decreased slightly over the same period in the areas that remained open to fishing. If organisms respond quickly to protection, initial rates of change should be greater than later rates. For the seven studies of reserves reviewed by Evans *et al.* (2006) that recorded temporal data, the rate of change for density, calculated as the ratio of the values at one time step divided by the value at the previous time step, was significantly greater for the first time step compared to all other time steps. Our recorded rapid increase in density and biomass at the shallower depths, compared with deeper sites could be attributed to relief from greater fishing pressure at these shallower depths. The most commonly used gears in the region are spear guns and hand lines, making shallow reefs more accessible to fishers. Overall, our results show a rapid, demonstrable, positive response of the targeted fish community to protection. This is a key element in maintaining support for community managed MPAs, particularly NTZs in rural subsistence fishing communities which have governance over their resources.

Acknowledgements
Thanks are extended to the communities of Ungakum, Kavulik, Lasigi, Malom, Silom and Dabanot for their partnership. Thanks to John Tagai Kuange and Roslyn Koi, and to John Aini of Ailan Awareness. Funding was provided by the David and Lucile Packard Foundation and the Commonwealth of Australia Department of the Environment and Heritage.

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Changes in reef fish biomass in Karimunjawa National Park: a test of the effectiveness of government gazetted marine parks in Indonesia

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Abstract. In June 2005, following extensive community consultation, national legislation was enacted to change the boundaries of zones that regulate fishing and other resource use in Karimunjawa Marine National Park in the Java Sea, Indonesia. The new park boundaries included five management zones ranging from no-take areas to open access. An initial increase in fish biomass in 2005 was not sustained and by 2007 there was no significant difference in mean biomass when compared to the initial surveys in 2004. Analysis by trophic groups suggested that the re-zoning was effective in protecting the biomass of many fishes. Indeed, only herbivorous fish biomass declined significantly within the park boundaries between 2004 and 2007; corallivore, omnivore and planktivore biomass increased while invertivore and planktivore biomass remained stable. However, similar patterns were evident outside the park boundaries, suggests other factors may be responsible for these patterns, such as a decline in effort throughout the region, perhaps as a consequences of a reduction in the number of people in the fishery. Some zones were more effective than others at protecting fish biomass. For example, increases in every trophic group, except herbivores, were recorded in the Core Zone and Tourism Zone. In contrast, most groups declined in the Protection Zone, perhaps as a result of a shift of fishing effort. Work in the park is continuing in an effort to identify the cause of these trends.

Keywords: Coral reef, Karimunjawa, management, marine protected area, , monitoring

Introduction

Between the years 2003-2004 the Wildlife Conservation Society (WCS) Marine Program Indonesia assessed the ecological condition of coral reef habitats, and evaluated socio-economic factors that constrain fisheries and conservation management in Karimunjawa National Park (KNP) in the Java Sea, Indonesia (Campbell 2006). In June 2005, following extensive community consultation, and using the data collected above, national legislation was enacted that changed the boundaries of zones to regulate fishing and the use of other marine resources in KNP. The park included five management zones ranging from no-take areas to open access. There were three Core Zones designated as no-take areas to protect three distinct reef habitats identified in the baseline surveys, 7 Protected Zones with limited access to resources, 7 Tourism Zones as no-take areas which nonetheless allowed for marine tourism, three Rehabilitation Zones (designed to allow degraded reefs identified in the baseline survey to recover and four Aquaculture Zones (for sea-weed farming, fish culture, etc.). The remaining areas within KNP were zoned Traditional Fisheries Utilization area (Utilization Zone) where fishing activity was unregulated.

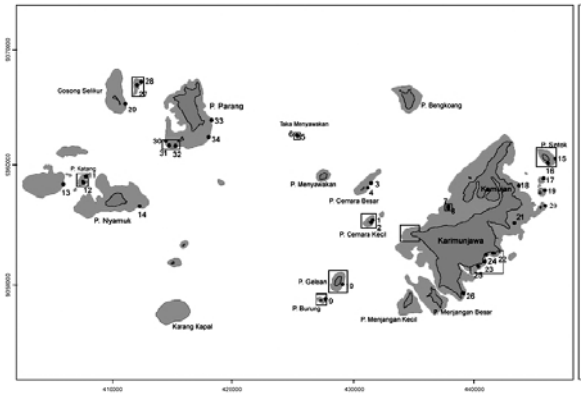

Figure 1. Map of Karimunjawa National Park and 43 survey sites within 5 management regimes.

As part of the ongoing evaluation of the new zoning scheme and as part of the adaptive management of KNP, monitoring has continued in the park. In total 43 survey sites were monitored during the transition time between the west and the east monsoon (April-May) in 2005, 2006 and 2007; The 2003-2004 baseline data (Ardiwijaya et al. 2007). plus surveys from areas outside of the KNP, which are open access, are included for comparison.

Materials and Methods

Fish abundance and biomass were collected using visual surveys at two depths (1 and 7 m) using two replicate 50m transects. A 5m width was used for fish whose Total length (TL) > 10cm and 2m for fish with TL $\leq$ 10cm. Fish TLs were classified into 9 size classes (see Fig. 3). Fish were identified to species following, Myers (1999) and Allen and Steene (1999). Fish data were analyzed using two-way ANOVA to test for significant differences between years and management regimes.

Fish from the family Pomacentridae (damselfish) were eliminated from the analyses to enhance precision since they are found in high and highly variable abundance within all survey sites. In 2005, there was no data collected outside the KNP. Consequently, the mean of sites of all other years was used as a baseline in ANOVA. All abundance and biomass values were $\log_{10}$ transformed.

Results

Mean fish abundance in the KNP fluctuated considerably among years and was significantly higher at the last census in 2007 when compared to 2004 (Fig.2a). In contrast, there was no significant increase in fish abundance outside KNP (Fig. 2a). Fluctuations in fish abundance in the KNP were driven mostly by changes in the Core Zone where a 100% increase in fish abundance occurred between 2004 and 2007 (Fig. 2a). Fish abundance did not vary significantly among years in any of the other zones (Fig. 2a). In contrast to abundance, fish biomass in KMP did not change between 2004 and 2007. An initial 20% increase in fish biomass in KNP in 2005 was not sustained. A significant increase in fish biomass in the Core Zone was counteracted by a significant decrease in the Protected Zone (Fig. 2b).

In general, an increase in abundance accompanied by decrease in biomass would suggest more small fish and/or less large fish. Yearly graphs of fish abundance in 9 size classes shows an increase in small (<10cm) and large fish (> 40cm) that was more than compensated for by an decrease in all size classes between 20 and 40cm (Fig. 3).

Benthic invertivore biomass was dependent on survey period and management zone (interaction term $F_{12,134}$=2.557; P=0.004). In the KMP as a whole there was no change in biomass of invertivores because significant increases in the Core and Tourism zones were counteracted by decreases elsewhere. (Fig. 4a).

Carnivorous fish biomass was dependent on survey period and management zone (interaction term $F_{12,134}$=2.267; P=0.012).

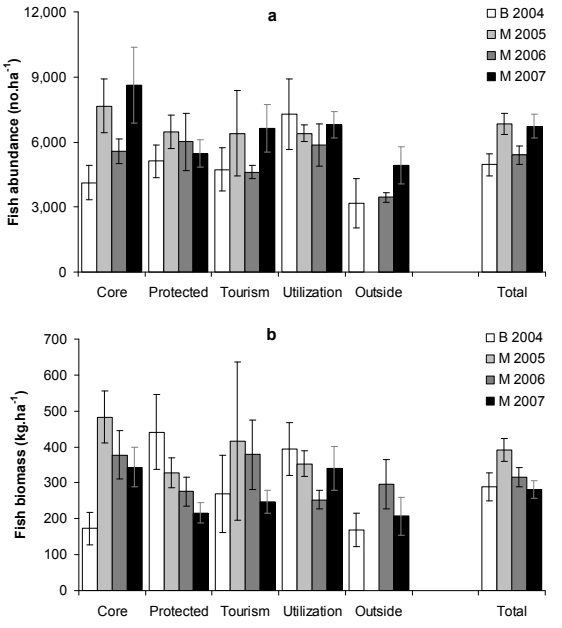

Figure 2. Mean (±SE) (a) abundance (no.ha^{-1}) and (b) biomass (no.ha^{-1}) of reef fish (excluding family Pomacentridae): comparison between management zones and survey periods.

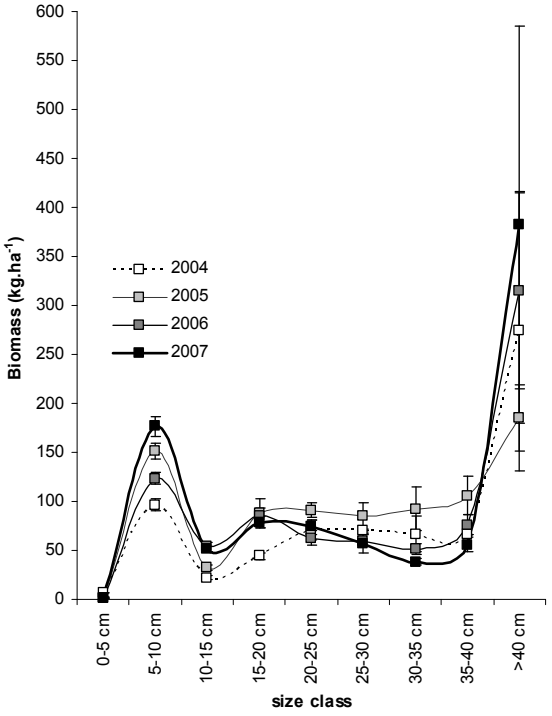

Figure 3. Mean (±SE) biomass (kg.ha^{-1}) of reef fish (Family Pomacentridae excluded): comparison between size classes.

In the KMP as a whole there was no change in biomass of carnivores because significant increases in the Core and Tourism zones were counteracted by decreases elsewhere (Fig. 4b). Similarly, the biomass

of carnivores outside the KNP in 2007 was not significantly different to that in 2004 (Fig. 2b).

Corallivorous fish biomass was dependent on survey period and management zone ($F_{12,134}$=1.886; P=0.044). In the KNP as a whole, biomass increased between 2004 and 2007 by nearly 100% driven mostly by large increase in the Core and Tourism Zone (Fig. 4c). A similar increase was apparent outside the KNP (Fig. 4c).

Herbivorous fish biomass was dependent on survey period and management zone ($F_{12,134}$=2.616; P=0.004). Large increases in the biomass of herbivores in the Core and Tourism Zones between 2004 and 2005 where not sustained and were more than compensated for by a large decrease in biomass in the Protected Zone between 2004 and 2007. The result was a 20% decline in herbivore biomass in KNP between 2004 and 2007 (Fig. 4d). Further analyses of the grazing parrotfish family Scaridae where performed to see how this family contributed to the patterns in biomass of herbivores. The patterns in biomass of the scarids among zones and years are very similar to those of the trophic group as a whole suggesting fluctuations in the abundance of this family is driving the patterns in the group (Fig. 5).

Omnivorous fish biomass was dependent on survey period and management zone ($F_{3,134}$=15.047; P<0.05). In the KNP as a whole, biomass increased between 2004 and 2007 by nearly 100%, driven mostly by large increases in the Core and Protected Zones (Fig. 4e). A similar, but not significant, increase was apparent outside the KNP (Fig. 4e).

Planktivorous fish biomass was dependent on survey period and management zone ($F_{3,134}$=9.562; P<0.05) In the KNP as a whole, biomass increased between 2004 and 2007 by nearly 200% driven mostly by large increases in the Core and Tourism Zones (Fig. 4f).

The omnivore trophic group was dominated by the family Pomacentridae which made up 70% of total abundance and 26% of biomass from all families. The planktivore trophic group was dominated by the family Caesionidae which made up 20% of total biomass (Fig. 6).

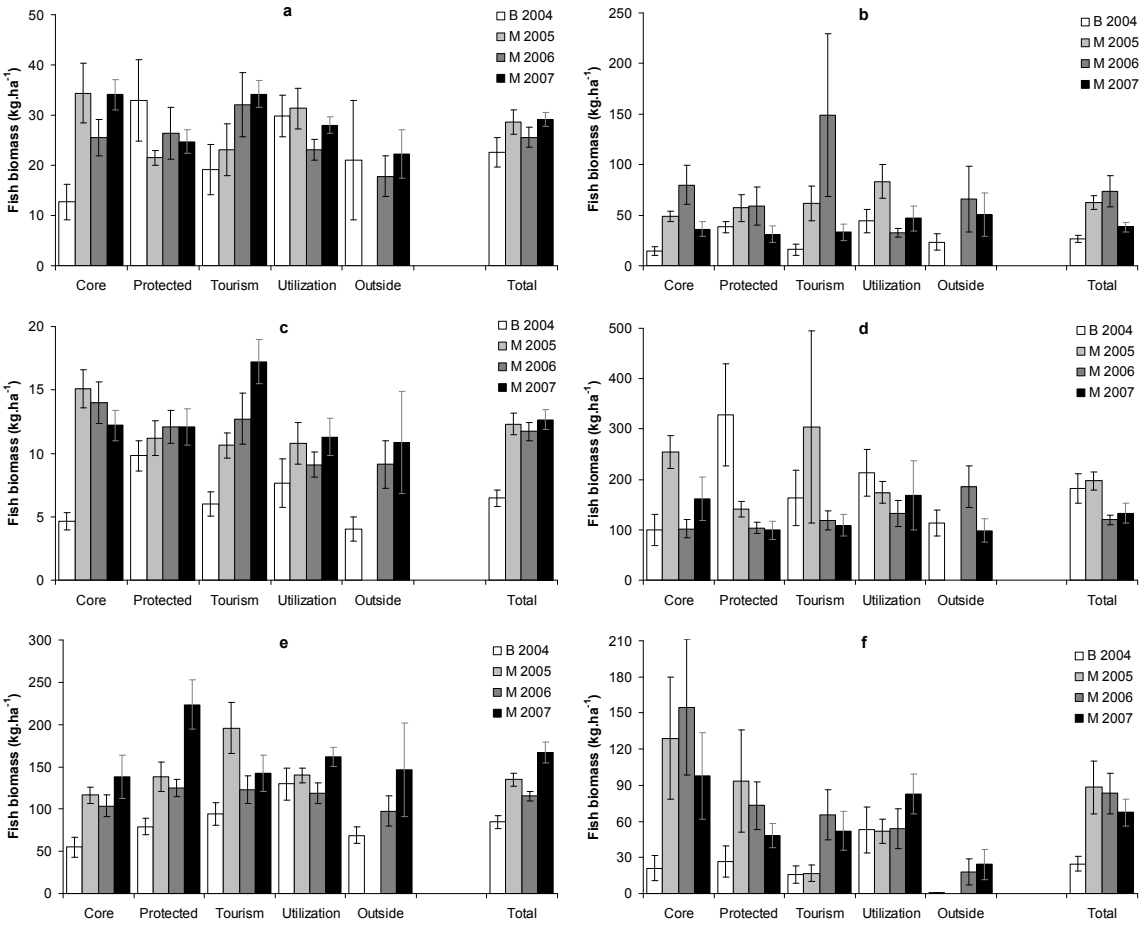

Figure 4. Mean (±SE) biomass (kg.ha^{-1}) of (a) benthic invertivore, (b) carnivore, (c) corallivore, (d) herbivore, (e) omnivore, and (f) planktivore: comparison between management zones and survey periods.

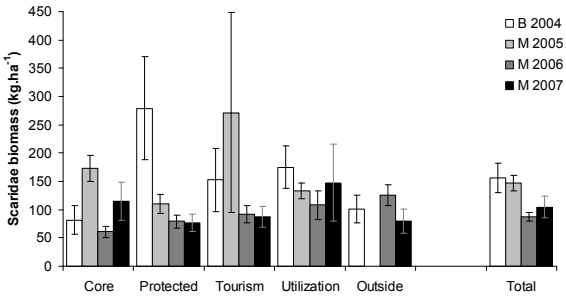

Figure 5. Mean (±SE) biomass (kg.ha^{-1}) of Scaridae (parrotfish): comparison between management zones and survey periods.

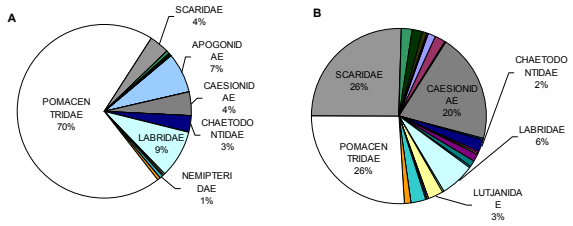

Figure 6. Composition of fish family by abundance (A) and biomass (B).

Discussion

Several interesting trends are apparent in these data. Slight increases in fish abundance has not delivered an increase in biomass, because the size structure of the assemblage now includes fewer fish in the size range from 20cm to 40cm. However, a number of trophic groups have increased in biomass since the re-zoning. In particular, the biomass of corallivores, omnivores and planktivores has all increased since the re-zoning in 2005. While these increases may not be of interest to fishermen, because few of these fish are targeted, they are potentially good news for the tourist industry. The corallivorous family, Chaetodontidae, are of particular interest to recreational divers. The biomass of invertivores and carnivores, the main target species for fisherman, remained stable within the KNP. Consequently, the re-zoning has been a partial success for these groups because it has prevented a decline in abundance while continuing to provide fishermen with access to the resource. Only the herbivores, driven mostly by a 20% decline in the biomass of scarids, have decreased in the KNP as a whole since the re-zoning. The loss of herbivores is of particular concern given the important role these fishes have in maintaining reef resilience (Hughes et al 2007). However, similar trends in the biomass of each trophic group outside the KNP suggest that factors other than the re-zoning may be causing these patterns. For example, fishing effort as whole may have declined in the region as fishermen, encouraged by the efforts of the local government and supported by international NGOs, move into other livelihoods.

Interestingly, some of the zones have been more effective than others in preserving or increasing biomass. In particular, the biomass of all trophic groups, except herbivores, has increased in the Core Zone. Similarly, the biomass of all groups, except herbivores and omnivores, has increased in the Tourism Zone. In contrast, the Protection Zone has been much less effective at protecting biomass, with large decreases in the biomass of most trophic groups. This suggests that fishing effort has increased in the Protected Zones as a result of fishermen shifting effort from the Core and Tourism Zones. Further research, documenting both fishing effort and location of catch will be required to explain the difference in the effectiveness of these Zones.

Fish biomass within KNP is lower than comparable MPA's in Indonesia, for example, the biomass per hectare is 50–100% lower than in similarly managed areas in Aceh (Fig. 7) suggesting the area may be over-fished and current fishing effort unsustainable over the long term.

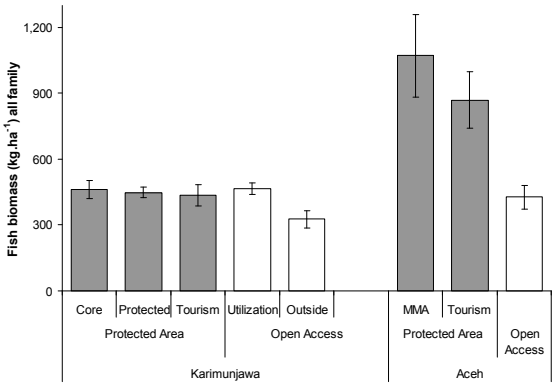

Figure 7. Mean (±SE) biomass (kg.ha^{-1}) of fish all family: comparison between Karimunjawa NP, and Aceh protected areas (Aceh Tourism and Aceh MMA).

The following recommendations have been made to the KNP authority

1. monitoring fish catch by documenting which fish are being caught and how many
2. evaluate fishing effort in each Zone and possibly restrict fishing gear especially nets
3. develop communication and awareness tools to enhance the perception of the benefits of marine parks in the local communities
4. patrol the KNP to measure compliance;
5. place more visible marking buoys to delineate Zones Boundaries to reduce non-compliance;

Acknowledgements

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Community sea tenure and the establishment of marine reserve networks in the Pacific islands.

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Abstract Recent studies call for the scaling-up and networking of local marine reserves for ecological effectiveness and resilience. Currently, the Pacific islands are witnessing an explosion in the number of community-based marine reserves. Village sea tenure is the foundation for the development of reserves in the Pacific and likely poses a distinct challenge to coordinated marine management and networking. We examine the Nguna-Pele Marine Protected Area network in Vanuatu to understand how sea tenure may act as a barrier or advantage to multi-community reserve networking. We found that networks of community reserves may be practical in the Pacific islands if they address a locally-defined need and build on existing collaborative governance arrangements. Networking frameworks must flexibly match local sociocultural contexts. We conclude that networks of reserves do not necessarily usurp management or ownership rights but rather may strengthen and legitimize community sea tenure regimes in the Pacific islands.

Key words: Marine reserve, customary marine tenure, Vanuatu, taboo, marine protected area network

Introduction

There has been a recent call by marine scientists to scale up global marine management focus from single marine protected areas to representative and connective networks of marine reserves (Hughes et al. 2005). In the Pacific islands however, governance and decision-making institutions most commonly operate at the community level (Berkes 2009). Non-centralized governance and ownership poses significant implementation challenges for effective and well-coordinated marine reserve networks in the Pacific islands (Lam 1998).

Though much is written on the diversity of marine tenure arrangements and management practices utilized in the Pacific islands (Bartlett et al. 2009), few studies have empirically examined the characteristics that allow for successful cross-tenure networking initiatives. We present the Nguna-Pele Marine Protected Area network in Vanuatu as a case study to further understand the conditions that enable a network of marine reserves to function across community tenure boundaries.

Methods

We carried out this research on the islands of Nguna and Pele from 2005-2007. A team of local researchers conducted interviews with over 80% of the population in six study communities. Qualitative data on local sea tenure arrangements was collected via focus groups, transect walks, document analysis, participant observation and key informant interviews.

Results

Marine Tenure in the Pacific islands

The most authoritative definition of customary marine tenure was forwarded by Ruddle et al. (1992) where: "customary" refers to a system that emerges from firmly traditional roots and has continuous and meaningful links with the past as it adapts to handling contemporary issues; "marine" refers to the system as dealing with coral reefs, lagoon, coast and open sea and including islands and islets contained in this overall sea space; and "tenure" refers to a social process of interacting activities concerning control over territory and access to resources.

More recently Aswani's (2002) definition removes reference to historical practices and defines sea tenure as "a situation in which particular groups of people have riparian entitlement to nearshore areas, and in which their entitlements to use and access resources are excludable, transferable, and enforceable, either conditionally or permanently."

Case study context: Nguna and Pele islands

Nguna and Pele are islands (17ºS and 168ºE) in the central Shefa province of the Republic of Vanuatu.

Each island is surrounded by fringing reefs, typically extending from 10-200m from the shoreline.

Human settlement and organization

The population of Nguna and Pele was just over 1100 people at the time of the last national census (Bakeo et al. 2000), and is spread unevenly among sixteen communities, ten of which are located directly on the coast. No village is more than three hours walk from any other.

Since the time of missionaization of the two islands in 1870, there have been several major changes to human settlement; massive depopulation forced some villages to converge and move towards the coast while others were abandoned (Don 1918). The most important impact missionaries had in the Nguna-Pele area was their reorganization of local social structures; a shift from a clan-line elected system of governance to a hereditary royal-family chiefly system.

Today a hereditary paramount chief presides over each village, assisted in governance duties by one or more lower chiefs. The function of the chief largely deals with preservation and promotion of custom (Bolton 1998). Day to day affairs and administration of the community however, falls to the democratically elected village council as is common in other parts of the country (Huffer and Molisa 1999). The council apparatus is a direct artifact of the influence of the Christian Church, and is often made up of several specialized committees.

Terrestrial resource use and ownership

In Vanuatu all land belongs to customary owners by decree of the national constitution. However, the definition of customary ownership remains undefined and has partly led to the current legitimization crisis facing the nation (Nari 2000). In Vanuatu, customary land owners are not always (or even usually) individuals, a trait that existing statutory land policy does not appropriately capture (Regenvanu 2008). Group right of access to natural resources is the norm in Pacific island societies, and is especially relevant and commonplace in the marine context (Ruddle 1998).

Encouraged by regional policies and exacerbated by investor-driven land acquisition (Hassall 2005; Regenvanu 2008), there is a legal trend away from group and toward private land ownership in Vanuatu. On Nguna and Pele, land areas and garden plots currently have relatively clear boundaries, though ownership lies with extended families rather than individuals. However, few legal deeds have been granted over parts of these two islands.

Village boundaries are perceived differently to family land parcels. A strong sense of village-ism and community patriotism exists on Nguna and Pele. Belonging to a community or village is a critical safety net, protecting against need by ensuring access to resources for even those without hereditary entitlements. Thus, the geographical boundaries of a community hold utilitarian importance on Nguna and Pele, even though most families have access rights to land outside their own community. Village boundaries on Nguna and Pele commonly originate from the top of a hill and follow a well defined physical feature, such as a creek or stone wall, to the coast.

Due to the uncodified nature of a "community", and the dynamic settlement context since missionization on Nguna and Pele, village boundaries are typically unclear and commonly under dispute. Most villages have areas of overlapping boundaries with adjacent communities. An unremarkable fact of life for most residents, these disputes have occasionally flared up into hostilities when benefits of tourism or infrastructural development were at stake.

Despite infrequent tensions between communities, the Nguna-Pele area is locally renowned for its area-wide historical collaboration. Villages on the two islands share a unique dialect as well as similar cultural and customary practices. Intermarriage among island villages ensures social connections remain strong. Another legacy of the Church's presence is the existence of robust, duel-island networks like the Nguna-Pele Presbyterian Session uniting the elders from each community, and the Nguna-Pele Council of Chiefs (NAPE).

Marine resource use and ownership

Few individuals or households on Nguna and Pele make an exclusive living from the sea, though most are involved in opportunistic fishing and reef gleaning. Some coastline villages, those on Pele for example, have a more frequent interface with sea resources than their inland counterparts. Household diet of sea resources is variable on both islands, dependent on the capacity and time allocated to fishing by family members.

In stark contrast to land ownership, areas of reef are not partitioned off for use by particular families or groups. Reefs are open for use by all members of a given community, with residents feeling little desire to exclude their fellow community members. Regular access to marine resources may be an important motivation for seeking clear and well-recognized community boundaries.

Community access to marine resources is generally allowed, as long as it fulfills only

subsistence or small-scale commercial needs. Major developments, such as shoreline construction or large-scale commercial harvest, require permission of the chief and the village council. It is not uncommon for a village member to make a monetary contribution to the council when undertaking a larger-than-usual harvest from village sea tenure area.

Marine boundaries are inherently difficult to define, and there exist a myriad of perceptions on the local extent of sea tenure areas on Nguna and Pele. Some residents consider that the outer edge of the reef constitutes the extent of a village's sea tenure. Generally however, it would not be tolerated for an outsider to fish within several hundred meters of the edge of a village's reef. Accordingly, many consider the sea tenure boundary to extend well into deep water, with some citing the horizon.

Marine governance

Exclusion of non-community members from using village resources is a *de facto* practice on Nguna and Pele. Permission must always be sought before reefs can be used by an outsider. Full time residency is the most common, but not exclusive, determinant of community membership. One role of the chief is to grant or revoke permission for outsiders to use a community's reef resources. This right does not imply that the chief owns those resources, but only that he or she is symbolically responsible for reef stewardship. In practice, the village council holds daily responsibility to define the uses, developments and restrictions within the community's sea tenure area.

The Nguna-Pele area may be one of the most advanced in Vanuatu in terms of its general consciousness of natural resource management issues. Each community's village council has established a conservation committee, whose primary responsibility is the maintenance and regulation of terrestrial and marine resources. Conservation committees propose and adapt the specific rules for use of the reef. These committees report to and follow the mandates of the village council, who in turn work under guidance of the paramount chief.

It is considered everyone's responsibility, including residents, the conservation committee, village council and chiefs, to comply with village marine regulations and report trespassers. Enforcement and surveillance is not difficult in most cases as reef areas are visible from the village. However there are cases on Nguna and Pele where the reef is located away from the settlement area, and trespassing events are likely more frequent.

Breaking the rules generally invokes a fine payable to the chief, which is then divided among the council and conservation committee. Infractions that involve non-community members however, are dealt with directly by the chief or village council of each respective community. Fines for non-community members are generally higher as these offenses are viewed as more severe.

Tension and ambiguity characterize the relationship between village, provincial and national-level marine policies. While higher levels of government recognize local governance institutions though Island Courts and Land Tribunals, village councils do not receive formal endorsement in national legislation. The Environmental Management and Conservation Act recognizes marine regulations set by village councils, but does not provide for enforcement or penalization.

Fisheries closures

The closure of all or part of a community's sea tenure area is widely practiced in the Pacific islands (Caillaud et al. 2004). Closures employed in the past by communities on Nguna and Pele were generally enacted in order to stockpile resources in preparation for a celebration or community event such as a wedding or chiefly ordination. Closure duration was commonly pre-decided, with enactment and rescindment independent of the quality or quantity of resources available on the reef.

In contrast, recently established marine closures in the Nguna-Pele and surrounding areas are being established with the clear objective of resource maintenance or conservation (Bartlett et al. 2009). Residents acutely perceive the decline of marine resources on their tenured reefs, and are aware of the global state of marine resources. A conservation ethic is real and very apparent on the islands of Nguna and Pele despite ongoing debates regarding its historical existence in the Pacific (see Foale and Manele 2004).

Contemporary community reserves in the region are small, typically less than 0.05km^2, and cover from 15-45% of the available sea tenured area (Bartlett et al. 2009). Strong sea tenure is the ultimately the foundation for the establishment of community fisheries closures.

Diverse marine reserve expressions

Although the goals and objectives of individual marine closures are similar, their form and expression is extremely diverse among communities on Nguna and Pele. We found three marine closure deigns common in the Nguna and

Pele area: permanent, rotational and periodically harvested reserves (Bartlett et al. 2009).

Permanent reserves are those in which the community indefinitely closes all harvest. Rotational reserves do not permit harvest during their term but are designed to be permanently opened in the not-too-distant future. Periodic reserves may allow infrequent and well-controlled harvest at any time, but generally not more than one or two times per year. Reserves (of all types) commonly restrict all species enclosed within, though reserve openings and harvests may target specific organisms.

The decision to implement a marine reserve is nearly always taken democratically. The village conservation committee commonly makes the suggestion in a village meeting, followed by public debate among residents. If a closure is approved, residents discuss potential locations and the type to be implemented. Final approval by residents to declare a reserve can take up to a year of detailed planning by the conservation committee. Once approved, a date is fixed so that notices can be sent to adjacent villages and customary preparations made.

Marine reserve declaration is attended by customary ceremony. The village chief, as the symbolic resource steward, proclaims the area off limits following the terms set by the community. The paramount chief will generally evoke ancestral protection over the area, deterring future rule-breakers and trespassers. Often, several pigs are killed at the site. Boundaries of the closed area are demarcated with recognizable custom objects. On Nguna and Pele, a *namele* palm leaf tied to a stake is the most commonly-employed marker, though large white stones, pig jaws and painted signs are also used.

Networking of community marine reserves

In 2002, four paramount chiefs established the Nguna-Pele Marine Protected Area Network in order to better coordinate the management of their village marine closures. They felt that village strategies were ineffective in isolation; resources were declining at an unprecedented rate. With the technical and ideological assistance of the Fisheries Department, the Vanuatu Environment Unit and international volunteer organizations, the network's membership has since expanded to included 14 communities on Nguna and Pele.

The Nguna-Pele MPA network is locally managed by representatives from each member community. Membership is open to all communities on the two islands. A full-time local manager, several part-time local staff and local village volunteers carry out the day to day activities of the network. These activities include cleanup campaigns, awareness talks, sea turtle tagging, eco-tourism, tree planting, social and ecological monitoring and management evaluation.

The Nguna-Pele MPA network does not hold ownership or enforcement rights over the sea tenured areas of any of its village members, nor does it dictate the type of closure and associated rules to be implemented. Rather, the network brings together representatives from each community to discuss, coordinate and collaborate on marine and terrestrial natural resource issues of area relevance. Networking enhances the political bargaining power area communities have with the national and provincial government in terms of the support they receive for marine management.

The benefits of networking are locally recognized, with villages often willing to adopt management strategies that may be most valuable to neighboring, 'downstream' communities. For example, a recent crown of thorns starfish outbreak was contained because area representatives planned and implemented a cross-tenure clean-up strategy. Discussions at Network meetings have also influenced the positioning of new reserves, particularly at boundaries with other communities, in order to create larger cross-tenure reserves. This island wide collaboration represents Vanuatu's first attempt at ecosystem-level conservation planning.

Discussion

Community tenure potentially limits the establishment of marine reserve networks in the Pacific islands. However, the Vanuatu case demonstrates that despite strong tenure, cross boundary decision-making and influence can be achieved through local networking. We have shown how village sea tenure is maintained as resource governance is simultaneously scaled up. Several factors have facilitated the success of the Nguna-Pele MPA network, some of which may be applicable in other Pacific island contexts.

On Nguna and Pele, marine resources are perceived to be in severe decline, likely driving the need and motivation for collaboration among area villages. A networking and conservation ethic exists because residents have directly observed the frustrating ineffectiveness of individual village actions. Though not acting completely altruistically, communities have shown they are willing to compromise and collaborate to obtain at least some reprieve from the locally perceived marine crisis. Inclusive and representative policies minimize the potential that the network serves the interest of some communities over others.

Networking in this case study is locally supported as it flexibly incorporates different types and expressions of marine reserves, and integrates these within chiefly and ceremonial practices. It does not discriminate or attempt to force a single approach or strategy. The Nguna-Pele MPA network's structure emulates previously existing island-wide organizations, eliciting local trust and participation. Networking is enhanced because all member villages speak the same language, are geographically proximate and share a historical trajectory. Management and implementation by local people helps engender the support of other island residents.

The costs of scaled-up governance are minimized at Nguna-Pele as individual reserve maintenance remains firmly situated at the village level. Communities are empowered, and their managerial roles expanded, through the retention and adaptation of tenure-based models of management and enforcement. In other words, reserve management (and associated cost) remains decentralized, while simultaneously allowing for scaled-up cross-tenure coordination. While not directly implementing or managing reserves, the Nguna-Pele MPA network has a powerful indirect influence on area-wide management scope and connectivity.

Conclusion

We know that marine commons dilemmas can be solved at small scales (Berkes 2006), with community conservation areas an increasingly popular approach (Berkes 2009). However, this case study demonstrates that scaled-up collaborative marine governance is achievable in the Pacific islands without compromising community marine tenure and associated management rights. Networks of marine reserves may be a real possibility in the region if they address a locally-identified need and build on existing management and governance arrangements. Flexible networking frameworks are required that reflect local sociocultural contexts. Ultimately, strong village tenure can be an advantage for establishing effective marine reserve networks, and may be critical for scaling up marine governance in a region with limited centralized governance capacity.

Acknowledgement

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An Ecological Correction to Marine Reserve Boundaries in the US Virgin Islands

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Abstract. Marine protected areas (MPAs) are important tools for management of marine ecosystems. While desired, ecological and biological criteria are not always feasible to consider when establishing protected areas. In 2001, the Virgin Islands Coral Reef National Monument (VICR) in St. John, US Virgin Islands was established by Executive Order. VICR boundaries were based on administrative determination of Territorial Sea boundaries and land ownership at the time of the Territorial Submerged Lands Act of 1974. VICR prohibits almost all fishing and other extractive uses. Surveys of habitat and fishes inside and outside of VICR were conducted in 2002-07. Based on these surveys, areas outside VICR had significantly more hard corals; greater habitat complexity; and greater richness, abundance and biomass of reef fishes than areas within VICR, further supporting results from 2002-2004 (Monaco et al., 2007). The administrative (political) process used to establish VICR did not allow a robust ecological characterization of the area to determine the boundaries of the MPA. Efforts are underway to increase amounts of complex reef habitat within VICR by swapping a part of VICR that has little coral reef habitat for a Territorially-owned area within VICR that contains a coral reef with higher coral cover.

Key words: Marine Protected Area, Coral Reef, Fishes, Overfishing

Introduction

Virgin Islands Coral Reef National Monument (VICR) is an MPA established by US President Clinton on January 17, 2001 under the Antiquities Act of 1906 (34 Stat. 225, 16 U.S.C. 431). These submerged lands consist of about 51 km^2 of marine habitat in five areas off the island of St. John, US Virgin Islands and are managed by the Secretary of the Department of the Interior through the National Park Service. VICR is comprised of many tropical marine habitats, including fringing mangroves, seagrass beds, nearshore coral reefs, a mid-shelf reef structure and deep water algal plains. Marine resources around St. John have declined dramatically over the past few decades (Rogers and Beets 2001, Beets and Rogers 2002). VICR was intended to enhance resources in the Virgin Islands and specifically in the Virgin Islands National Park (VINP), which was established by Congress in 1956 and expanded to include submerged lands in 1962. This new Monument roughly doubles the area in and around St. John now under the jurisdiction of the National Park Service (NPS). Provisions within the Presidential Proclamation prohibit all extractive uses with the exception of fishing for a coastal pelagic species, blue runner (*Caranx crysos*) south of St. John and bait fishing in a small area within the Coral Bay component of VICR (Fig. 1). In addition, boat anchoring is prohibited in VICR, except for emergency or authorized administrative purposes. The NPS, the National Oceanic and Atmospheric Administration (NOAA), and the US Geological Survey (USGS) initiated a joint project in 2002 to develop a baseline characterization of species and their associated habitats within and outside VICR to assess changes within the ecosystem. The project objectives have evolved to monitor the efficacy of the marine reserve and adjacent areas.

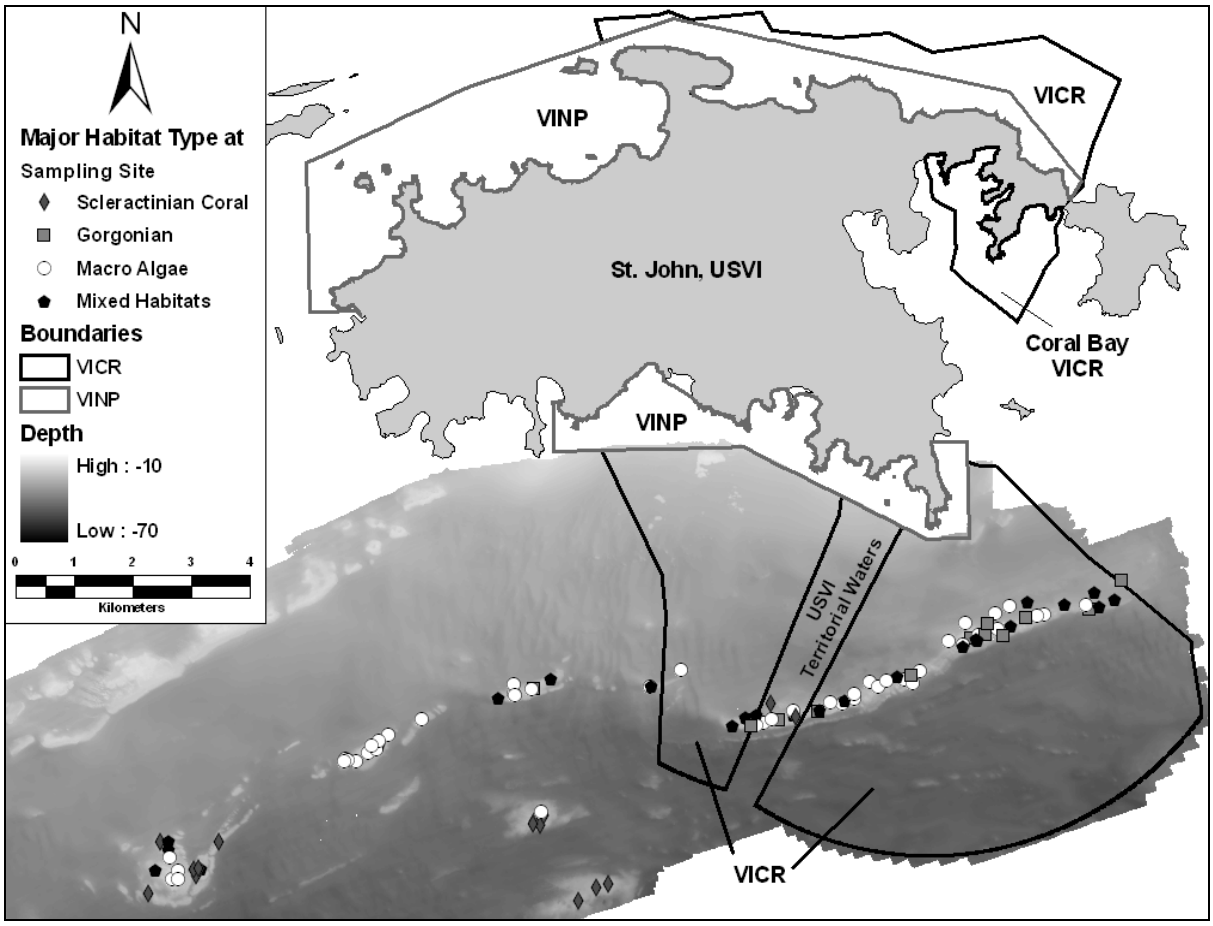

Figure 1. Boundaries of Virgin Islands National Park (VINP) and Virgin Islands Coral Reef National Monument (VICR). Symbols represent survey locations and major habitat types defined at each location.

Material and Methods

The mid-shelf reef is unique for St. John, owing to its relatively deep (17-35m) coral reef ecosystem and relatively high percent live coral cover and topographic complexity. Not all of the mid-shelf reef area is contained within VICR because the MPA was delineated based on legal parameters of the Submerged Lands Act that define federal waters. As a result, VICR is bisected by a wedge of submerged land that extends out from shore from privately owned lands that are not included in the VINP. Additionally, the mid-shelf reef extends to the west of St. John and south of St. Thomas.

Sampling sites were randomly selected inside and outside of VICR within the coral reef habitat of the mid-shelf reef. Modeled bathymetry derived from NOAA depth soundings was used as a surrogate to identify areas of potential coral reef habitat. Additional bathymetry data for water depths ranging from 20-100m for the area south of St. John (Fig. 1) were provided from multibeam surveys (2004-05) off the NOAA ship R/V *Nancy Foster*. Visual reef fish surveys were conducted annually in July from 2002 to 2007 and associated fine-scale habitat data were also obtained during the surveys. Fish abundance and size at each location were obtained on SCUBA via visual belt transect surveys (25x4m). A second diver assessed habitat quality (coral cover) and complexity (rugosity) within a 15-m diameter circular sampling area nested within the belt area. Both of these parameters are based on a visual estimation as defined by the rapid habitat assessment protocol with rugosity scaled from 1 to 3 (low to high).

Results

Habitat Comparisons

Although there was no significant difference in depth between management strata ($P>0.05$), there were differences between the benthic habitats inside vs. outside VICR (Table 1). Rugosity and live coral cover were greater outside VICR compared to inside VICR ($P<0.05$). The extent of hard bottom habitats outside VICR was greater than inside VICR. No sites within VICR were classified as having live scleractinian coral as a dominant biotic component (Fig. 1).

However, gorgonian cover was significantly higher inside VICR than outside (Table 1).

Table 1. Mean (±SD) benthic habitat characteristics inside and outside VICR along the mid-shelf reef south of St. John (2003-07). P is the probability of a significant difference between inside and outside VICR. Asterisk (*) indicates significant difference (P<0.05).

Habitat characteristic	Outside VICR mean (± SD)	Inside VICR mean (± SD)	% Differ-ence	P value
Abiotic				
Depth (ft)	87.76 (8.76)	87.03 (8.86)	-0.8	0.555
Rugosity	2.21 (0.66)	1.92 (0.81)	-15.1	0.007*
Hard bottom (%)	85.76 (9.654)	81.21 (23.89)	-5.6	0.07
Sand (%)	10.53 (7.97)	13.07 (16.59)	19.4	0.161
Rubble (%)	3.7 (4.09)	4.88 (11.49)	24.2	0.322
Biotic				
Live scleractinian coral cover (%)	18.41 (19.37)	4.46 (6.4)	-312.8	<0.001*
Gorgonian cover (%)	11.46 (7.87)	19.72 (14.8)	41.9	<0.001*
Macroalgal cover (%)	46.28 (18.83)	42.45 (21.84)	-9.0	0.18

Fish Assemblage Comparisons

Fish assemblage characteristics (species richness, numerical density, and biomass) differed significantly between management strata (Table 2), with all significantly greater outside VICR. Among the three habitat types represented both inside and outside VICR (gorgonian, macroalgae, and mixed coral), fish assemblage characteristics were not significantly different (Two-way ANOVA, P>0.05) for all.

Table 2. Comparison of mean (±SD) fish assemblage characteristics inside and outside VICR along the mid-shelf reef south of St. John (2002-07). P is the probability of a significant difference between inside and outside VICR. Density and biomass ln(x+1) transformed for statistical analyses. Asterisk (*) indicates significant difference (P<0.05).

Fish assemblage characteristic	Outside VICR mean (± SD)	Inside VICR mean (± SD)	% Differ-ence	P value
Species richness	27.05 (5.87)	23.0 (0.44)	-0.18	<0.001*
Density (indiv/100m^2)	307.92 (23.52)	215.79 (13.71)	-0.43	0.003*
Biomass (kg/100m^2)	10.49 (8.78)	8.21 (6.7)	-0.28	0.01*

Comparisons Among Major Fish Families (2002-2007)

- Total parrotfish biomass was 42% higher outside than inside VICR (Z=4.33, P<0.001). These species are important grazers on the reef
- Economically important groupers had 44% higher biomass outside VICR relative to inside (Z=2.33, P=0.02; Fig. 2)
- Fish biomass has increased inside VICR since 2005 primarily due to an increase in piscivores; however piscivores have declined outside over this same time period (Fig. 3)

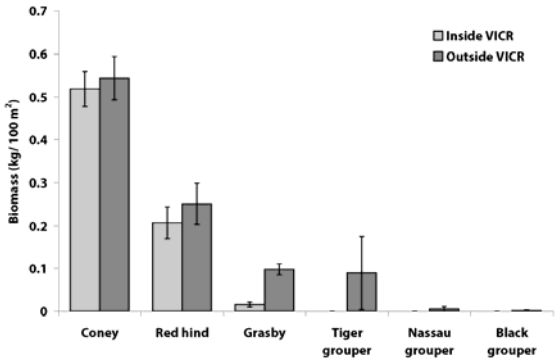

Figure 2. Mean biomass of an economically important fish family, groupers (Serranidae), inside and outside VICR (2002-07). Error bars represent standard error of the mean.

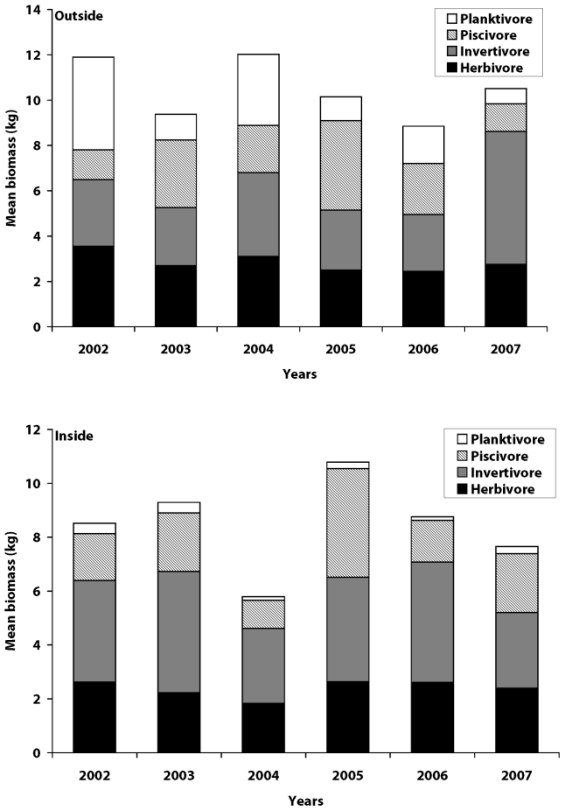

Figure 3. Biomass of trophic guilds outside (top) and inside (bottom) VICR.

Conclusions and Next Steps

The establishment of VICR was a politically driven process based on an exception in the Submerged Lands Act of 1974. As a result, much of the optimal coral reef and fish habitat on the mid-shelf reef is located both in the wedge and to the west of VICR.

Nevertheless, establishment of this MPA does provide for protection of the resources and may lead to resource enhancement over time, especially if an ecological correction can be applied.

While reef sites outside of VICR cannot be considered for inclusion an ecological correction can be made by exchanging the eastern, less biologically rich portion of VICR for the wedge of Territorial Sea that currently bisects VICR on the south side of St. John (Fig. 4a).

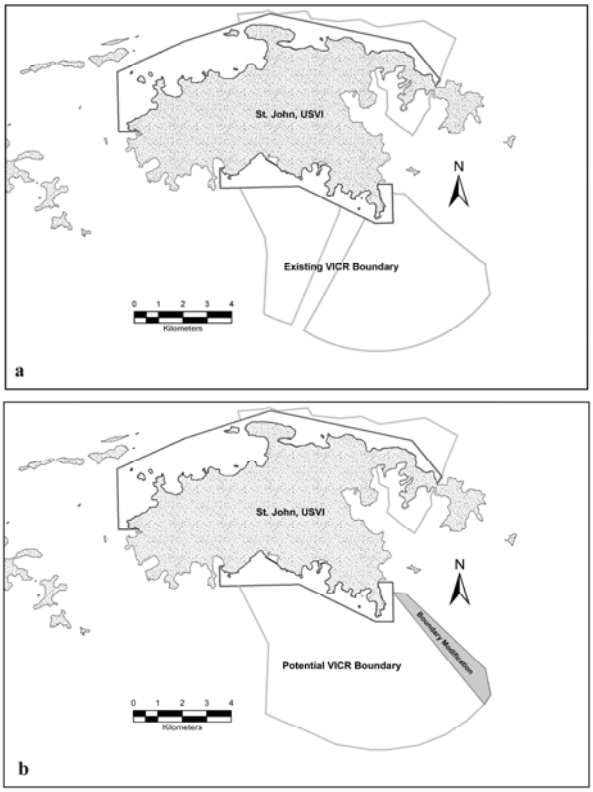

Figure 4. a) Existing VICR boundary and b) potential modification of VICR boundary along the south shore of St. John, USVI.

This process requires agreement from both Territorial and Federal governments in order to occur. The process was initiated before the election of a new VI Governor which delayed progress. The new Governor and the local Department of Planning and Natural Resources, along with many fishermen, have expressed support for the exchange. A recent meeting with the VI Delegate to Congress has resulted in a letter from the Governor to the Secretary of the Department of Interior requesting that negotiations begin for the exchange. As the wedge is already within the authorized boundaries of VICR, this should facilitate the boundary adjustment (Fig. 4b). The major issue will be an appraisal of the submerged lands to be exchanged, since no precedent exists for appraising deep water coral reefs and algal plain.

Inclusion of the mid-shelf reef currently located within the wedge will increase the amount of coral reef and fish habitat in the VICR and provide greater opportunity for resources in this MPA to recover from past harvest of resources and other stressors. This will also eliminate the concern of fishermen that they may be violating VICR regulations and will enhance enforcement of those regulations by NPS law enforcement rangers.

Acknowledgements

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Biological Criteria for Protection of U.S. Coral Reefs

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Coral reef ecosystems are threatened by natural stressors, human activities, and natural stressors exacerbated by human activities. Under the U.S. Clean Water Act, States and Territories may guard against anthropogenic threats by adopting water quality standards based on biological, physical, and chemical criteria. The condition of biological communities is a more dependable water quality standard than are physical and chemical attributes because living organisms are responsive to effects of low-level, chronic, cumulative, interacting point- and non-point source pollution. The President's Ocean Action Plan directed the U.S. Environmental Protection Agency (EPA) to develop biological assessment methods and tools for evaluating the health of coral reefs so that States and Territories could more easily establish biological water quality standards, including descriptions for designated waterbody uses and biological criteria (biocriteria). Biocriteria are qualitative or quantitative thresholds of biological condition necessary to sustain the designated uses. Rigorous biological assessments are needed to identify metrics that reflect biological characteristics and are responsive to a gradient of human disturbance, and to generate defensible long-term monitoring programs. Implementation of biocriteria for freshwater ecosystems has forged a process that can be adopted for coral reefs. EPA is fostering development of coral reef biocriteria through research, evaluation, and collaboration.

Key Words: bioassessment, biocriteria, Clean Water Act, coral reefs, U.S. EPA

Coral reef ecosystems are declining worldwide, threatened by a variety of human activities including polluted runoff from agriculture and land-use practices, over-fishing, ship groundings, coastal development and climate change, as well as by natural stressors such as tropical storms, bleaching and disease (Knowlton and Jackson 2008). Concern over the existing and impending threat of climate change on coral reef communities has been expressed (Richardson and Poloczanska 2008), but local anthropogenic sources also contribute directly to reef decline and can exacerbate climate change impacts (Mora 2008). Local managers have little control over climate change, but regional policies and local decisions for waterbody activities and watershed land use could substantially reduce anthropogenic stresses.

In the United States and its dependent territories there are a variety of management approaches and opportunities for reducing anthropogenic stresses to coral reefs. The National Oceanic and Atmospheric Administration (NOAA), the National Park Service (NPS) and U.S. Fish and Wildlife Service (FWS) function as natural resource custodians for coral reefs and commonly establish Marine Protected Areas (MPAs) to protect habitat from physical damage and to restrict fishing (e.g., no-take zones). MPAs can be reserved by federal, state, territorial, tribal, or local laws or regulations; federal MPAs have been established by NPS, FWS, and NOAA (National Marine Fisheries Service, National Estuarine Research Reserve System, and National Marine Sanctuary Program). MPAs restrict human activities in particular areas in order to promote non-consumptive and non-destructive uses of natural and cultural resources, such as coral reefs. Even outside coral reef MPAs, responsible fishery management may be promoted through fishing restrictions and habitat protection. Managers may employ additional tools, such as mooring buoys and informational programs to protect reefs from physical damage and pollution.

Recently, natural resource managers have initiated programs to support coral reef resilience, or potential for recovery from climate change events (e.g., Keller 2008). Some resilience goals include:

- Assurance that resilient species and habitats are well-represented and replicated throughout the coral reef ecosystem, and in particular, inside protected areas
- Protection of critical areas, such as fish spawning aggregations and nursery habitats
- Preservation of connectivity among reefs and their associated habitats.

Finally, the U.S. Environmental Protection Agency (EPA), under aegis of the Clean Water Act (CWA), is authorized to protect coral reefs and coastal zones from sediment, nutrients, contaminants or microorganisms introduced into water bodies from human activity. This protection can be achieved through a variety of programs to reduce sewage effluent, industrial contaminant releases, agricultural pesticides, nutrient applications, and riverbed stability. However, the full weight of the CWA has not been directly applied in coral reef protection. The CWA creates the opportunity for States, Tribes, and Territories to implement enforceable water quality standards based explicitly on the biological condition of coral reef communities.

U.S. Clean Water Act

The Clean Water Act (CWA) is the primary federal law governing water pollution control in the United States. The objective of the CWA is to restore and maintain the chemical, physical and biological integrity of the Nation's waters. There are several regulatory vehicles in the CWA to curb pollution, and these indirectly protect coral reefs. For example, the CWA provides controls for point (Section 402) and nonpoint (Section 319) pollution sources, and a strong foundation for attainment of aquatic life uses (Section 303). However, there is even greater potential to protect coral reefs by focusing designated uses and water quality standards explicitly on coral reef conditions.

The CWA requires that States, Tribes, and Territories define chemical, physical and biological expectations for water resources (i.e., water quality standards), monitor conditions regularly, and report whether those expectations are being met. Water quality standards include 1) designated uses, which reflect goals for the water body, 2) numeric and narrative criteria to achieve the designated uses, and 3) antidegradation policies to prevent deterioration of high-quality waters (Karr and Yoder 2004).

States and Territories establish designated uses based on the physical, chemical, and biological characteristics of the water body, its geographical setting, and at their discretion scenic qualities and economic values as well. Waterbodies can be protected for public water supply, for protection and propagation of fish, shellfish, and wildlife, and for recreational, agricultural, industrial, and navigational purposes. Designated uses can also be written to specifically protect coral reef ecosystems by further defining the desired biological attributes. This is particularly compelling for those jurisdictions with generic designated uses.

For example, some jurisdictions may have simply adopted water classes for designated uses: *Class A* waters might cover many of the generic uses described above (i.e., navigation, agriculture, drinking water, aquatic life protection) and would be considered higher quality waters in need of more stringent criteria than *Class B* and *Class C* waters. More specific language could be added to these legally binding narratives to characterize the biological goals for coral reef ecosystems. This could include the need to maintain the structure and function of coral reefs similar to the natural community or a condition similar to minimally impaired reference sites. Designated uses for coral reef protection could include specific goals, such as '*protection of reef fish spawning aggregations'*, or '*protection of coral taxa richness and community structure*'. While revision of designated uses can be a time-consuming process, it is well worth the benefits to coral reef protection. The Biological Condition Gradient (BCG), as described by Davies and Jackson (2006) can be applied to the development of designated uses for coral reefs. Physical, chemical and biological criteria establish thresholds, qualitative or quantitative, to benchmark the desired water quality necessary to support the designated uses. Biological criteria (biocriteria) are perhaps the most relevant to coral reefs, not only because they integrate multiple cumulative stressors, but also because biological measurements resonate with managers and stakeholders. Biocriteria, codified under CWA Section 304a, represent the agreed upon condition, attributes and services that society seeks to maintain. Biocriteria specific to coral reef condition are a necessity if valued reef attributes are incorporated into designated uses.

Biocriteria and several other aspects of the CWA rely on biological assessments (bioassessments), which directly measure the condition of one or more taxonomic assemblages (e.g., corals, fish) and the chemical and physical attributes that support those assemblages. Since the community of plants and animals reflect the underlying health of the waterbody in which they live, assessments of species richness, species composition, population size, and trophic

composition of resident biota are the most direct measures of biological integrity (Karr 1991).

To implement quantitative (numeric) biocriteria, States and Territories must determine the biological conditions that sustain the designated uses, select measurements that reflect the relevant biological conditions (e.g., range, variability and responsiveness to human disturbance), and establish a defensible long-term monitoring program (Jameson et al. 2001). Coral reef biologists have a strong history of bioassessments and monitoring, and have developed biological indicators that might ultimately serve as biocriteria metrics, including:

- live coral coverage
- proportion of species tolerant to stressors of concern (e.g. pH, nutrients, temperature, sediments, metals, pathogens, etc.)
- number of species (taxa richness)
- proportion of exotics
- prevalence of disease
- percent recent mortality
- coral recruitment

Waterbody Attainment or Impairment

The CWA provides several different regulatory vehicles to curb pollution and thereby protect coral reefs. Section 305(b) of the Clean Water Act requires each State to prepare a biennial report on the quality of its waters. A 305(b) report describes the extent to which waterbodies support their designated uses. The report also identifies the pollutants or stressors causing impairment of designated uses and the sources of these stressors (e.g., wastewater treatment plants, agriculture, or industrial plants). EPA transmits the individual 305(b) reports to Congress along with a summary report on the Nation's water quality prepared using the 305(b) information. A well-prepared, scientifically sound 305(b) report can be beneficial to coral reef managers in these ways:

- The 305(b) report is a public information tool documenting State/Tribal/Territorial actions to protect waterbodies; it increases the visibility of the water quality programs.
- The report can call attention to special issues such as loss of critical fish spawning aggregation areas or coral reef habitat.
- The process offers an opportunity for State/Territorial technical staff to coordinate assessments and data management for shared waters.

Section 303(d) of the Clean Water Act requires each State and territory to periodically prepare and submit a list of specific waterbodies that currently violate or have the potential to violate water quality standards, including designated uses and numeric or narrative biocriteria. These lists must prioritize the waterbodies for development of Total Maximum Daily Loads (TMDLs). TMDLs identify the maximum amount of a pollutant that a waterbody can receive and still safely meet water quality standards, and allocate that amount among various sources (e.g., nutrient loads could be allocated between agriculture and sewage systems).

Comprehensive Strategy to Address Impaired Waters

It is a clear benefit of a biocriteria program that each monitoring cycle automatically triggers a regulatory decision (e.g., 303(d) listing requires priority rankings and development of TMDLs). Potential regulatory actions that managers can implement include:

- Increase the scope of National Pollutant Discharge Elimination System (NPDES) permits to incorporate bioassessments and biological thresholds
- Establish marina pumpout facilities and no discharge zones
- Implement new permit requirements (i.e., require that commercial and recreational boaters hold pollution discharge permits covering deck and hull cleaning, fueling, trash management, and graywater discharges)
- Require dischargers to evaluate wastewater reuse and recycle options or install more advanced technology

There are also non-regulatory, Best Management Practices that managers can choose to implement, such as:

- Agricultural (runoff control, grass/riparian buffers, manure management, conservation tillage, etc.)
- Urban and residential (upgrade sewer and water infrastructure, xeriscaping, pesticide management, riparian buffers, etc.)

Coral reef protection calls for integrated, collaborative management. Clear, shared objectives and improved coordination of programs are a necessity. States and Territories can adopt a watershed approach to managing coral reefs, where community officials, industries, environmental groups, and citizens throughout the watershed basin jointly identify the problems and develop priorities and actions to address them. By effectively managing watersheds, we can protect both the health of coral reefs and the people who depend on them. The CWA regulatory vehicles, including biocriteria, provide watershed-based management options to protect coral reef ecosystems that complement existing natural resource management.

EPA's Biocriteria Outreach

While the responsibility for establishing water quality standards lies with the States and Territories, EPA is providing support to define conservation and management needs, refine designated uses, develop bioassessment procedures and monitoring strategies, establish reference conditions and biological thresholds, differentiate anthropogenic from natural causes of change, and identify causes of adverse effects.

Over the last 20 years, EPA has developed a body of work to support implementation of biocriteria protective of freshwater and estuarine ecosystems (e.g., EPA 1996, 1998, 2000). Ongoing research (http://epa.gov/bioindicators/coral/) will help develop comparable opportunities for coral reefs.

Current Efforts of States and Territories

The U.S. Coral Reef Task Force has identified the need for local action to reduce key threats to coral reefs and called for the development of Local Action Strategies (LAS) in each of the seven states and territories that possess significant coral reef resources. While no LAS specifically addresses biocriteria, each is focused on improving water quality and coral reef condition through better understanding of the links between land-based pollution and coral reef health; improving compliance and enforcement of laws, rules, and regulations related to construction and land development permits; and implementation of best management practices to reduce pollution from agricultural and residential areas. Specific biocriteria-related activities of the States and Territories include:

American Samoa and the Commonwealth of the Northern Mariana Islands (CNMI): New narrative biocriteria have been proposed that include coral species richness (Houk et al. 2005). CNMI received an EPA Wetlands Program Development Grant to support the development of numeric biological criteria for coral reefs, and American Samoa is in the process of evaluating four measures as potential coral biocriteria: 1) coral diversity per unit area, 2) total coral diversity, 3) coral community evenness, and 4) a benthic substrate ratio.

Florida: An established quality assurance process is in place to ensure that data for Clean Water Act purposes are properly and consistently collected. Florida's Department of Environmental Protection has begun to identify and evaluate protocols that could be applied as biocriteria, beginning with EPA's Stony Coral Rapid Bioassessment Protocol (Fisher 2007).

Hawai'i: EPA is funding a wetlands grant to devise biocriteria based on the Hawai'i Coral Reef Assessment and Monitoring Program (Jokiel et al. 2004).

US Virgin Islands (USVI): Designated uses are being revised to include more specific biological attributes, and EPA is assisting USVI's Department of Planning and Natural Resources to design an integrated monitoring program to assess coral reef condition (Fisher et al. 2008).

Summary and Conclusions

Thus several US jurisdictions now recognize that the CWA provides a suite of powerful regulatory tools that can be applied to protect coral reefs. The process to establish a biocriteria program has been vetted in both freshwater and estuarine programs, and can also be applied to coral reefs. Coral reef biologists have knowledge and expertise, as well as a strong history of monitoring and assessment. They have also developed biological indicators that could ultimately serve as biocriteria metrics for supporting assessment of coral condition relative to the set standards, and for implementing regulatory or non-regulatory corrective action. Development of biocriteria is not easy, but many of the tools are available. With strong collaborative effort, we can successfully develop and apply biocriteria to protect U.S. coral reefs.

The development and implementation of coral reef biocriteria can also be incorporated into Water Quality and Coral Reef Programs outside the US and its territories. For example, the Coral-based Indicators of Changes in Water Quality on Nearshore Coral Reefs of the Great Barrier Reef (Cooper and Fabricius 2007) may be candidates for biocriteria to determine if Australian and New Zealand waters meet their beneficial use "Maintain health of aquatic ecosystems". In addition, the Healthy Mesoamerican Reef System Initiative has developed a suite of ecological indicators for coral reef ecosystems that may serve as biocriteria for Mexico, Belize, Guatemala, Honduras, Nicaragua and El Salvador (McField and Kramer 2006).

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Macrobenthic diversity reaction to human impacts on Maceió coral reefs, Alagoas, Brazil

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Abstract. Brazilian coral reefs have many endemic species. In the state of Alagoas, coral reefs are abundant, but different human impacts occur when the reefs are located in urban settings. This study compared the diversity of macrobenthic organisms on five coral reefs on the urban coast of the city of Maceió, which are subject to different human impacts. Semi-quantitative estimates of the abundance of live specimens at each station on these reefs consisted of visual records obtained during five full low-tide periods from 2005 to 2007. Fifty taxa of macrobenthic organisms were analyzed, using cluster analysis (UPGMA, Bray-Curtis). The Jatiúca (50 species) and Ponta Verde (50 species) coral reefs were similar, with 0.2 for cluster analysis; both sites are impacted only by harvesters of seafood. The Pajuçara (48 species) and Amores (47 species) coral reefs were similar, with 0.4 and 0.5, respectively; human impacts at these sites also include boat anchoring. Piscina Natural coral reef had the fewest macrobenthic species (13), with 0.7 for cluster analysis; human impacts on this reef include tourist activities and boat anchoring. These results call for stricter control of human impacts on coral reefs situated on the urban coast, and demonstrate that macrobenthic organisms can be useful indicators for monitoring coral reefs.

Key words: Brazilian reefs, invertebrate reefs, urban reefs, human impacts

Introduction

The Atlantic region is divided into four reef provinces; the Brazilian province is quite distinct because of its small number of coral species. Many of these species are endemic (Veron 1995), and are thus important for reef conservation. The first reports on coral reefs from Brazilian coast were by Laborel (1965, 1969a, b), who characterized scleractinian corals and hydrocoral zonation for different reefs along this coast. He also mentioned the impacts by many lime kilns, locally called "caieira," which exploited coral to obtain whitewash to correct the soil pH for sugarcane agriculture. The northeast coast of Brazil has the largest concentration of reef ecosystems, starting from the Manuel Luiz Banks in the state of Maranhão (Amaral et al. 2007) to the Abrolhos area on the south coast of the state of Bahia (Hetzel and Castro 1994). On the Abrolhos reefs, the species diversity and zonation patterns of hermatypic corals on two fringing reefs was studied by Pitombo et al. (1988), and the colonization and growth of crustose coralline algae were characterized by Figueiredo (1997). Castro and Pires (2001) noted that relatively little information exists about Brazilian coral reefs. Shallow-water scleractinian corals and Zoanthidea from the Coroa Grande reefs in the state of Pernambuco were characterized by Neves et al. (2002) and those from Itacolomi reefs in southern Bahia were characterized by Castro et al. (2006), who demonstrated differences in cover composition and species abundance along the northeast coast of Brazil. The marine sponge fauna of the Pernambuco coast is similar to that of other areas on the northeastern Brazilian coast, according to Muricy and Moraes (1998).

Belém et al. (1986) described environmental problems on coral reefs along the Brazilian coast, and called attention to some of the human impacts. Some reef ecosystems are being degraded by tourism in several areas, where there is a high intensity of activities because of the large flow of people and the lack of adequate planning. The environmental impacts caused by human activities on reef ecosystems were studied in the Abrolhos area (Coutinho et al. 1993, Leão et al. 1994, Leão 1996) and Fernando de Noronha island (Maida et

al. 1995), demonstrating that environmental degradation was linked to uncontrolled tourist activities.

On the coast of the state of Alagoas, environmental aspects and a general characterization of coral reefs, including faunal diversity, were presented by Sovierzoski and Correia (1995). Coral reefs are abundant on the Alagoas coast, and in some areas, fringing reefs are located near the beach line where the top of the reef platform is exposed during low tides. On these reefs, different human impacts occur, principally at urban sites, which are often visited by hundreds of people including local seafood harvesters and tourists, and are also used for boat anchoring (Correia and Sovierzoski 2005). The composition of the fauna associated with the different phytal areas was studied by Santos and Correia (1994, 1995, 2001), who demonstrated the great diversity of invertebrate species. On the Alagoas reefs, some information about species diversity and ecological aspects were described for the phylum Porifera (Sarmento and Corrreia 2002; Cedro et al. 2007). The first record of a living catenicellid bryozoan in the Atlantic Ocean was given by Vieira et al. (2007), from coral reefs on the Maceió coast.

The present study compared the effect of human impacts on the macrobenthic diversity in the intertidal zone, for five coral reefs situated on the urban coast of Maceió city, Alagoas.

Materials and Methods

The study area is situated on the urban coast of Maceió city, capital of the state of Alagoas, northeastern Brazilian coast, where there are many coral reefs (Fig. 1).

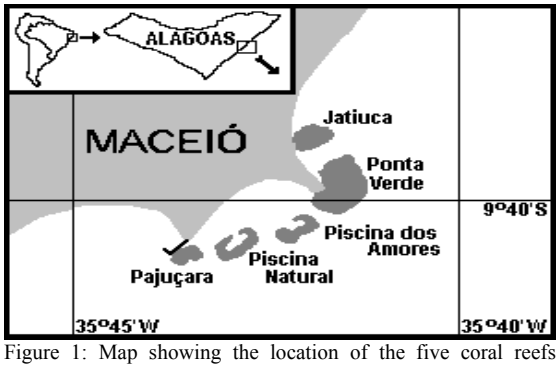

Figure 1: Map showing the location of the five coral reefs investigated on the urban coast of Maceió city, Alagoas, Brazil.

The present research was carried out in the intertidal zone on five urban coral reefs on the Maceió coast, where different human impacts occur. Some of these fringing coral reefs are located adjacent to the beachline, such as Jatiúca coral reef (9º39'38''S / 35º42'05''W), Ponta Verde coral reef (9º40'10''S / 35º41'30''W) and Pajuçara coral reef (9º42'18''S / 35º43'36''W). Two other coral reefs, Piscina dos Amores coral reef (9º41'18''S / 35º42'24''W) and Piscina Natural coral reef (9º41'48''S / 35º42'10''W), are situated at different depths in the offshore zone (Fig. 2).

All five coral reefs have the platform top exposed during low tide, where some macrobenthic organisms occur. However, the species are more concentrated in the reef pools, which are up to 2 meters deep. The reef borders have countless irregularities, with small entrances and some inlets with an accumulation of sand in the low areas, and strong wave and current action.

Semi-quantitative estimates of the abundance of live specimens on five transects, each 10 metres long, were made for the intertidal zone and in the reef pools of the five coral reefs. These estimated were based on an exhaustive compilation of visual records made during five full low-tide periods from 2005 to 2007. Fifty taxa of macrobenthic organisms were analyzed: 17 Porifera, 10 Cnidaria, 9 Echinodermata, and 14 macroalgae, because these organisms were the most common on these reefs. These macrobenthic organisms were selected because they are most common on the reef intertidal zone at Maceió coast. The macrobenthic analyses were based on the percentage cover for each species, showing the colonization intensity.

Figure 2: Aerial view of the coral reefs investigated on the urban coast of Maceió city, Alagoas, Brazil: (A) Jatiuca, (B) Ponta Verde, (C) Piscina dos Amores, (D) Piscina Natural, (E) Pajuçara.

On the urban coral reefs of Maceió, different human impacts occur: some areas are frequently visited by hundreds of people during low tide, principally in summer. The principal human

activities are local seafood harvesters, boat anchoring, and tourists, with different intensities depending on the number of people and boats per day during low tide that were transformed percentage occurrence. The Bray-Curtis index and UPGMA were measured for cluster analysis, to determine the similarity between macrobenthic organisms on the five reefs on the urban coast of Maceió city.

Results

There was an inverse relationship between the level of macrobenthic diversity and the level of human impacts at these sites. On the Jatiúca and Ponta Verde coral reefs, all of the 50 species of macrobenthic organisms were found (17 Porifera, 10 Cnidaria, 9 Echinodermata and 14 macroalgae). On the Pajuçara coral reef, 48 species were found; and 47 species were recorded for Piscina dos Amores coral reef. The Piscina Natural coral reef differed the most, with only 13 species, mostly macroalgae (Table 1).

Different intensities of human impact were recorded on the five coral reefs on the urban coast of Maceió city. All these coral reefs are usually visited by hundreds of people, such as local seafood harvesters, boat anchoring, and tourists, with differing intensities. On the Jatiúca and Ponta Verde coral reefs, few human impacts were observed, mostly seafood harvesters. On the Pajuçara and Piscina dos Amores coral reefs, human impacts were also caused by anchored boats. The Piscina Natural coral reef was subject to the highest level of tourist activities and boat anchoring, which occurs intensely year-round, principally in summer and vacation periods (Table 2).

Table 2: The intensity of human impacts on the five coral reefs investigated on the urban coast of Maceió city, Alagoas, Brazil. Legend: XXXX 100%, XXX < 75% , XX < 50% , X < 20%

	CORAL REEFS				
HUMAN IMPACTS	Jatiuca	Ponta Verde	Pajuçara	Piscina dos Amores	Piscina Natural
Seafood harvesters	XXX	XXX	XXX	XXX	XXX
Boat anchoring	X	X	XX	XX	XXXX
Tourists activities	X	X	X	XX	XXXX

Jatiúca and Ponta Verde coral reefs were more similar, with distance 0.2 in cluster analysis, where there was high diversity and low human impacts intensities were found. The Pajuçara and Piscina dos Amores coral reefs were included in the same cluster, with 0.4 and 0.5, but had fewer species and more human impacts. The greatest difference was observed on the Piscina Natural coral reef, with 0.7 for cluster analysis; there were few species, and the human impacts were caused by many different activities, as well as the presence of tourists (Fig. 3).

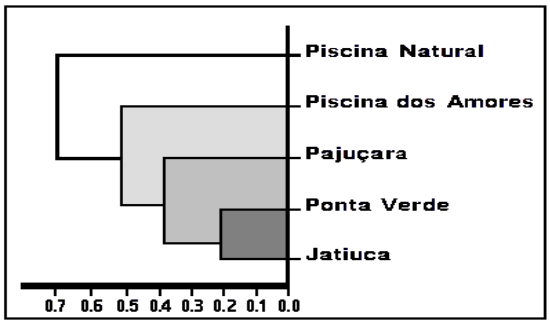

Figure 3: Cluster analyses on the five coral reefs investigated on the urban coast of Maceió city, Alagoas, Brazil.

Discussion

These results demonstrate that the diversity of macrobenthic organisms can be useful for monitoring coral reefs on the Brazilian coast, to discern the various levels of human impact. The coral reefs on the urban coast of Maceió city have many problems caused by human activities. Previous reports have described the different human impacts on coral reefs of the Brazilian coast, such as the studies of Laborel (1969a, b), who mentioned the whitewash industry, commonly referred to as "caieira", although this activity no longer exists.

However, this study indicates that there are more problems related to human impacts associated with tourist activities, which cause serious damage to the Maceió coral reefs. At all these sites, the scleractinian corals were most sensitive to different impacts among other macrobenthic organisms analyzed.

The results demonstrated differences in composition and species abundance along the northeast coast of Brazil, compared to information from Manuel Luiz Banks in the state of Maranhão (Amaral et al. 2007) and the Abrolhos area (Pitombo et al. 1988). However, on the Maceió coral reefs, the cnidarian species cited in this study are common on other coastal coral reefs in southern Pernambuco (Neves et al. 2002) and Bahia (Castro et al. 2006), as are the sponge

species on the coast of the state of Pernambuco (Muricy and Moraes 1998) and on the Maceió coast (Cedro et al. 2007).

On the impacted coral reefs, macroalgae occupy the substrate (Lirman 2001, McCook et al. 2001), because they grow faster than the invertebrate species such as corals and sponges. The evidence of this competition was found principally on the highly impacted Piscina Natural coral reef, with algae dominance and few invertebrate species.

Many other coral reefs areas along the Brazilian northeast coast have similar problems caused by human impacts (Belém et al. 1986, Coutinho et al. 1993, Maida et al. 1995). In all these reef areas, such as the Pisicna Natural coral reef, the highest impact level is caused by tourist activities, because they are visited by large numbers of people, principally during the summer vacations.

These observations illustrate the need for stricter control of human activities on urban coral reefs on the Maceió coast, with monitoring activities and restricting access, specifically in the coral reef areas where tourist activities occur.

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Table 1: Macrobenthic diversity on the five coral reefs investigated on the urban coast of Maceió city, Alagoas, Brazil.
Legend: XXXX 100%, XXX < 75% , XX < 50% , X < 20% , - 0%

	TAXA	CORAL REEFS				
		Jatiuca	Ponta Verde	Piscina dos Amores	Piscina Natural	Pajuçara
	PORIFERA					
1	*Amphimedon viridis*	XXX	XXX	XX	-	XX
2	*Amphimedon compressa*	XXX	X	X	-	X
3	*Chalinula molitba*	X	XX	X	-	X
4	*Chondrilla nucula*	XXX	XXX	XX	-	XX
5	*Chondrosia collectrix*	X	XX	X	-	-
6	*Chondrosia reniformis*	X	XX	X	-	X
7	*Cinachyrella alloclada*	XX	XXX	XXX	-	XXX
8	*Cinachyrella apion*	X	X	-	-	X
9	*Cliona celata*	XX	XX	X	-	XXX
10	*Cliona varians*	XX	XX	X	-	XX
11	*Haliclona curacaoensis*	XX	XX	X	-	XX
12	*Haliclona manglaris*	XX	XX	X	-	X
13	*Haliclona melana*	XX	XX	X	-	X
14	*Niphates erecta*	XX	X	X	-	X
15	*Spirastrella hartmani*	XX	X	X	-	X
16	*Tedania ignis*	XXX	XXX	X	X	XXX
17	*Tethya maza*	X	XX	X	-	XX
	CNIDARIA					
18	*Agaricia agaricites*	XXX	XX	X	-	X
19	*Favia gravida*	XX	XXX	X	-	X
20	*Mussismilia hartii*	X	X	X	-	X
21	*Mussismilia hispida*	X	X	X	-	X
22	*Palythoa caribaeorum*	XX	XXX	X	XX	XX
23	*Palythoa variabilis*	X	XX	X	-	X
24	*Porites asteroides*	XXX	XX	X	-	X
25	*Porites branneri*	XXX	XXX	X	-	X
26	*Siderastrea stellata*	XX	XXX	XXX	-	X
27	*Zoanthus zociathus*	XXX	XXXX	X	X	XX
	ECHINODERMATA					
28	*Diadema antillarum*	XX	X	X	-	X
29	*Echinaster echinophorus*	X	X	-	-	-
30	*Echinometra lucunter*	XXXX	XXXX	XX	X	XX
31	*Eucidaris tribuloide*	XX	X	X	-	XX
32	*Lytechinus variegatus*	X	X	X	-	XXX
33	*Linckia guildingii*	XX	XX	X	-	XX
34	*Ophioneris reticulata*	X	X	X	-	X
35	*Ophioderma cinereus*	XX	X	X	-	X
36	*Ophiotrix angulata*	X	X	X	-	X
	MACROALGAE					
37	*Acanthophora* sp	X	X	-	X	X
38	*Amphiroa fragilissima*	XX	XXX	XX	X	XX
39	*Bryopsis sp*	XX	XX	X	-	XX
40	*Caulerpa mexicana*	XX	X	X	-	X
41	*Caulerpa racemosa*	XX	XX	X	X	XX
42	*Dictyosphaeria* sp	XX	XX	X	X	XX
43	*Dictyota cevicronis*	XX	XX	X	-	X
44	*Enteromorpha* sp	XX	XX	X	X	X
45	*Halimeda opuntia*	XX	XXXX	XX	X	XX
46	*Gelidiella* sp	XX	XX	X	X	X
47	*Gelidium* sp	X	XX	X	XX	X
48	*Padina* sp	XX	XX	X	-	X
49	*Sargassum cymossum*	XX	XX	X	X	XX
50	*Ulva lactuca*	X	X	X	-	XXX
	Total species	50	50	47	13	48

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The U.S. Coral Reef Task Force: A Model for Fostering Coral Reef Management at National to Local Scales

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This report assessed the strengths and weaknesses of U.S. Coral Reef Task Force (USCRTF) efforts to mobilize U.S. coral reef conservation at local to national levels. The success of the USCRTF has been its ability to bring together government entities with diverse and potentially conflicting mandates to identify common national goals and increase on-the-ground efforts addressing local and national coral reef conservation priorities. The USCRTF includes 12 U.S. Government agencies, seven states and territories, and three Freely Associated States. Through this broad membership, the USCRTF is uniquely situated to promote a holistic, ecosystem-based approach to coral reef conservation. The USCRTF developed the first *U.S. National Action Plan to Conserve Coral Reefs* and the *National Coral Reef Action Strategy* to define and direct its overarching goals, objectives, and activities. The national goals were further refined into place-based Local Action Strategies (LAS) to address six priority threats. The LAS provide a framework for USCRTF members to address national goals through on-the-ground action. While the USCRTF has significantly advanced U.S. coral reef conservation efforts, some key challenges include the inability to set clear conservation goals, targets, schedules and performance metrics, an imbalance of time spent on developing conservation plans rather than focused effort on implementing on-the-ground action, and little power/progress to stem the tide of government sponsored actions impacting reefs.

Key Words: U.S. Coral Reef Task Force, Local Action Strategies, coral reefs, conservation

Introduction

The United States Coral Reef Task Force (USCRTF) was established in 1998 by Presidential Executive Order 13089 to lead, coordinate, and strengthen U.S. government actions to conserve coral reef ecosystems; both domestic and international. The Executive Order recognizes the value of coral reef ecosystems and directs the U.S. Government agencies to work independently "to ensure actions they authorize, fund, or carry out will not degrade the conditions of such ecosystems." To fulfill this mission, the USCRTF develops national strategies, targeted initiatives, and new partnerships to strengthen stewardship of the coral reef ecosystems in the United States and internationally.

USCRTF membership includes leaders from twelve Federal agencies, seven states, territories, and commonwealths, and three Freely Associated States (See Fig. 2). To implement the policies and requirements of the Executive Order, the USCRTF provides a forum for collaborative planning, priority-setting, partnership building, and action among federal agencies, state and territorial governments, and non-governmental partners. The unique success of the USCRTF has been the ability to bring together government entities with diverse mandates and expertise to identify national goals and foster work at the regional and jurisdictional level that addresses both local and national priorities for coral reef conservation.

National Level Collaboration

The USCRTF developed the first U.S. *National Action Plan to Conserve Coral Reefs* (2000) and the *National Coral Reef Action Strategy* (2002) to identify and develop an implementation strategy for national coral reef conservation goals and objectives (see Fig. 1). To implement the *National Action Plan* and *National Action Strategy*, the USCRTF has supported a variety of actions to reduce adverse impacts to coral reef ecosystems, raise the profile of coral reef issues and increase awareness of the threats facing these valuable ecosystems. Some examples include:

The USCRTF has committed to produce comprehensive digital maps of all U.S. shallow-water

Figure 1: ***U.S. National Coral Reef Action Strategy***
13 Goals for Addressing Threats to Coral Reefs:

THEME 1: Understand Coral Reef Ecosystems—
Goal 1: Create comprehensive maps of all U.S. coral reef habitat.
Goal 2: Conduct long-term monitoring and assessments of reef ecosystem conditions.
Goal 3: Support strategic research to address the major threats to reef ecosystems.
Goal 4: Increase understanding of the social and economic factors of conserving coral reefs.

THEME 2: Reduce the Adverse Impacts of Human Activities—
Goal 5: Improve the use of marine protected areas to reduce threats.
Goal 6: Reduce adverse impacts of fishing and other extractive uses.
Goal 7: Reduce impacts of coastal uses.
Goal 8: Reduce pollution.
Goal 9: Restore damaged reefs.
Goal 10: Improve education and outreach.
Goal 11: Reduce threats to coral reef ecosystems internationally.
Goal 12: Reduce impacts from international trade in coral reef species.
Goal 13: Improve coordination and accountability.

(less than 30 meters) coral reef habitats, and to characterize priority moderate-depth (30-200 meters) reef systems. Completed digital map products cover 6,340 square kilometers of U.S. shallow-water coral reef ecosystems.

The *National Action Plan to Conserve Coral Reefs* calls for the development of a report on the state of U.S. coral reef ecosystems. These reports, The State of Coral Reef Ecosystems in the United States and Freely Associated States, produced in 2002, 2005, and 2008, characterize the condition of shallow-water coral reef ecosystems based on quantitative results of assessment and monitoring activities conducted by Federal, state, territory, commonwealth, non-governmental, private, and academic partners.

The USCRTF works to build capabilities to address such ecosystem-scale threats as disease, bleaching, and other sources of mass mortalities. To help meet this objective, the Coral Disease and Health Consortium (CDHC) was created in 2002 to provide coastal and ocean managers with scientific understanding and tools to help address coral health issues and mitigate degradation. The CDHC is a network of field and laboratory scientists, coral reef managers, and agency representatives devoted to understanding coral health and disease. It is cross-disciplinary, highly collaborative, and completely voluntary. Over 100 partners—including the Environmental Protection Agency, Department of the Interior, NOAA, other Federal agencies, academia, non-profit organizations, and industry—contribute their time and expertise

In 2005, coral reefs in the wider Caribbean suffered a widespread and severe bleaching event, which resulted in extensive coral death in much of the region. The USCRTF passed a decision item in November 2005 to mobilize efforts across the Caribbean to monitor, assess, and research short- and long-term impacts of the 2005 warming and bleaching event. The USCRTF Bleaching Committee coordinated the efforts of NOAA, NASA, the Department of the Interior's USGS and NPS, other government agencies, non-governmental organization partners, university researchers, and local managers.
The USCRTF acknowledges that marine protected areas (MPAs) are an important coral reef management tool, and has taken measures to incorporate this tool into their marine resource management efforts. The USCRTF called for strengthening the Nation's existing network of MPAs with particular attention to increasing the number of "no-take" marine reserves—areas where extractive uses are prohibited—and designing coordinated networks of coral MPAs in U.S. waters and other areas to ensure the long-term viability, ecological integrity, and sustainable use of coral reefs. The USCRTF has taken measures to assess how effectively this tool has been applied and USCRTF members have taken action to use MPAs to protect coral reef ecosystems.

Linking National Goals to Local Action – Local Action Strategies
While the USCRTF as a whole continues to make progress on the national goals, through a parallel and complementary effort these goals were further refined to focus on local, place-based issues. In 2002, the USCRTF developed place-based Local Action Strategies (LAS) which are designed to address priority threats at the local, jurisdictional level. The LAS provide a framework for USCRTF member agencies to identify and address priority threats and additional local needs, connect local priorities to national goals, coordinate Federal agency actions with local management of reef resources, and increase collaboration and resources to implement conservation actions. This approach allows the USCRTF members to tailor their goals and activities to address local issues and support on-the ground action.

Through the LAS process, the following priority threat areas were identified from the U.S. *National Action Plan to Conserve Coral Reefs* (2000):

- over-fishing,
- land-based sources of pollution,

- recreational overuse and misuse,
- lack of public awareness, and
- climate change, coral bleaching and disease.

Further, three jurisdictions used the LAS process to identify additional specific conservation issues and concerns for local action. These issues are:

- Maritime Industry and Coastal Construction Impacts – Florida,
- Invasive Species – Hawaii, and
- Overpopulation – American Samoa.

A *Status Report on Implementation of the Local Action Strategies* covering the fiscal years 2002 to 2006 identified approximately 760 projects across the seven jurisdictions and reports that the LAS have generated $25 million from numerous government and non-governmental sources applied to project implementation to date. Local agencies have leveraged hundreds of thousands of additional finds through volunteer services and in-kind resources, including time and skills, which have not been quantified in the total amounts of support received.

Local Place-Based Conservation Action

The ten USCRTF jurisdiction members span a vast geographic area, each with their own distinct set of issues, priorities, and stakeholder communities. The LAS framework has been specifically designed and used in each of the U.S. State, territory, and Commonwealth jurisdictions to address a particular set of issues and to meet the needs of the local community.

Two LAS examples follow, from Florida and Hawaii, which illustrate the value of and flexibility inherent in the LAS process.

Florida

Florida's LAS process serves as a model for the flexibility and specificity that the LAS structure allows. To meet a very specific management challenge, Florida developed the *Maritime Industry and Coastal Construction Impacts (MICCI*) LAS. Florida, more than any of the other USCRTF jurisdiction partners, experiences intense coastal use, development, shipping activity, and has an active network of commercial ports. This LAS is, therefore, intended to address activities such as vessel anchoring and groundings, infrastructure installation (e.g., cables, pipelines, and outfalls), beach nourishment, and dredge and fill operations in and around coral reefs and coastal habitats that can adversely affect these sensitive ecosystems. The key goals and objectives of the MICCI Focus Team are to:

1. Evaluate existing coastal construction and marine industry practices and their potential (or documented) impacts, and develop alternative or innovative methods and processes that significantly minimize or eliminate those impacts to marine habitats.
2. Compile and/or develop appropriate procedures for response to reef impacts, and reef restoration and monitoring.
3. Review existing regulations and suggest modifications or promote new regulations (as appropriate).
4. Encourage compliance with existing regulations.

Lack of awareness and knowledge of construction practices that can minimize these impacts is a significant source of the continued degradation of the southeast Florida reef ecosystem. Impacts associated with coastal construction projects and activities conducted by contractors unaware of the presence and vulnerability of the reef could be reduced through a combination of strategies to increase awareness and by refining some practices to better protect coral reef habitats.

Hawaii

In Hawaii, the *Climate Change and Marine Disease Local Action Strategy* illustrates how the LAS process can provide a mechanism to apply adaptive management among and between various regions and jurisdictions. *Hawaii's Climate Change and Marine Disease Local Action Strategy*, in part, is a good example of how a jurisdiction has taken existing programs and activities that have proven successful elsewhere and adapted them to meet the specific needs and to accommodate the stakeholder community in Hawaii. In designing this LAS, Hawaii has adopted and adapted monitoring programs and protocols that have proven successful in both Australia and Florida. In addition, Hawaii has taken advantage of an existing network of coral disease experts, the Coral Disease and Health Consortium, to strengthen and enhance their existing capacity for coral disease research, training, and management activities. (Report citations can be found in the reference section.)

As these two examples show, the LAS process provides the USCRTF members with a mechanism to translate national level goals into action for locally driven priority needs. Additionally, at the local level there is opportunity for flexibility and adaptive management schemes in the design and implementation of the LAS.

National Priorities Implemented Locally

While the USCRTF has taken advantage of its unique membership working directly with jurisdictions to

identify and address their priority coral reef conservation issues, the USCRTF continues to coordinate action at a national level. The USCRTF provides a forum for Federal agency members to explore how their diverse mandates, authorities, and programs can be used in concert to reach common conservation goals.

One such example is a U.S. Department of Agriculture (USDA) and NOAA collaborative partnership with Puerto Rico in the Jobos Bay National Estuarine Research Reserve. USDA's Conservation Effects Assessment Project is an effort to quantify environmental effects and benefits of conservation practices on upland agricultural lands. NOAA contributes the scientific research and monitoring data of the near-coastal waters to help make the connections between land-based conservation practices, near-shore water quality parameters, and sediment chemistry within the Bay. This is the first such project in the tropics and is an initial effort to link USDA land conservation practices with NOAA coral ecosystem monitoring activities to determine ecological impacts from land-based sources of pollution.

The USCRTF aims to promote more of these national programmatic partnerships effectively directed towards on-the-ground conservation actions and solutions.

Challenges and Weaknesses

While the USCRTF provides a forum for collaboration across Federal and local agencies, the USCRTF has faced a number of significant challenges in its attempts to increase coordinated action to conserve valuable coral reef ecosystems and the communities that depend on them. At the core, the Executive Order that established the USCRTF is not technically binding in any way, and therefore, provides no explicit authority for the USCRTF as a body. In addition, the USCRTF has no budget or independent source of funding to help promote and facilitate collective and coordinated action but rather is dependent upon individual agency budgets that can be applied to coral reef related activities. The USCRTF serves as an effective planning and coordination body at some levels; however, one of the greatest challenges has been efforts to identify conservation goals and targets, and the establishment of performance metrics at national to local levels to track progress towards the *National Action Plan* goals. The lack of such conservation targets impedes the ability to assess the overall impact the USCRTF or member actions have for conserving coral reef ecosystems.

These fundamental limitations are compounded by the challenges inherent in working cooperatively across many diverse partner mandates, authorities, and interests at the national and local level. For example, the initial process to develop LAS was not necessarily an easy or smooth transition among members to restructure their organizational framework to better link national goals to local action. This, combined with varying levels of capacity to develop and implement LAS has resulted in differing degrees of success across jurisdictions and throughout the life cycle of LAS projects.

Finally, at the National level, the USCRTF and its members are not taking full advantage of the Federal authorities and the potential role they could play in reducing impacts on reefs from threats directly or indirectly impacting coral reef systems. Action also needs to be stepped up across the USCRTF to better implement the Executive Order charge for Federal members "to ensure actions they authorize, fund, or carry out will not degrade the conditions of such ecosystems."

Conclusion & Discussion

Partnership for strategic and collaborative action

The USCRTF has created a range of effective partnerships and in turn, developed much needed capacity at the national and local level. Through the USCRTF, Federal agencies are directed to work together to address, in a collective and strategic way, the threats to coral reef ecosystems and to lead, coordinate, and strengthen U.S. Government actions to conserve coral reef ecosystems. To fulfill this mission, the USCRTF developed national strategies, targeted initiatives, and new partnerships to strengthen stewardship of the coral reef ecosystems in the United States and around the world.

In the ten years since its inception, the USCRTF has taken a variety of actions to reduce key threats and adverse impacts to coral reef ecosystems. It has led the development of national and local strategies to conserve coral reefs, increased collaboration among federal agencies and jurisdictions in coral reef regions, and helped its members launch new actions to protect and manage reef ecosystems. In particular, the real and tangible value of the USCRTF is through its efforts to create a forum for and to build upon the following:

- Foster Partnership
- Strengthen Coordination
- Increase Financial and Technical Resources
- Promote Efficiency
- Improve Effectiveness

However, despite these efforts, many reefs continue to be degraded or destroyed by unsustainable fishing, destructive fishing practices, land-based pollution, and coastal development, among other natural and human-induced impacts. In addition, climate change is having a variety of impacts on coral reef ecosystems, including increasing acidity of our oceans and the loss of corals from bleaching and disease linked to increased sea surface temperatures. Continued efforts for coral reef conservation are dependent upon a host of partners and require a multi-faceted approach. It is essential that the scientific understanding, tools and political will at all government levels exists to take effective action to address the threats these reef systems face. The USCRTF must seek to better utilize individual member authorities, programs, and expertise at the national and local level to respond more effectively to those threats that the USCRTF has the capacity to address.

The USCRTF continues to build on this strong foundation to develop and advance new and innovative opportunities for collaboration among Federal agencies and coral reef jurisdictions. Urgent, coordinated action and a recognition that greater authority is needed to stem further decline of these valuable coral reef ecosystems.

Figure 2: U.S. CRTF Members

Federal Agencies
U.S. Department of Commerce, NOAA
U.S. Department of the Interior
U.S. Agency for International Development
U.S. Department of Agriculture
U.S. Department of Defense
U.S. Department of Homeland Security
U.S. Department of Justice
U.S. Department of State
U.S. Department of Transportation
U.S. Environmental Protection Agency
National Aeronautics and Space Administration
National Science Foundation

States and Territories
Commonwealth of the Northern Mariana Islands
Commonwealth of Puerto Rico
State of Florida
State of Hawaii
Territory of American Samoa
Territory of Guam
Territory of the US Virgin Islands

Non-Voting Members
Federated States of Micronesia
Republic of the Marshall Islands
Republic of Palau

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Finding the right fit for ecosystem-based management in the Philippines

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Abstract. Spatial scales for ecological marine protected area networks do not necessarily fit into existing governance frameworks in the Philippines. The Philippines adopts a highly decentralized approach to coastal management including marine protected area (MPA) management. Each local government authority exercises management powers and responsibilities over their coastal zone which is measured from the shoreline to 15-km seaward of municipal waters. The local government initiatives have resulted in the establishment of over 300 MPAs in Central Philippines to improve marine habitats and enhance fishery resources, with only 20-30% of MPAs effectively managed with sustainable fishery benefits. With the increasing number of MPAs, the need for joint MPA management efforts in order to increase management effectiveness as well as protect areas beyond MPA boundaries has arisen. Thus, a definitive governance structure has been sought within the fisheries ecosystem scale following the ecosystem-based management approach. This paper will present the management strategies, outcomes and lessons of the Local Governance for Coastal Management Project for the Management of Coral Reefs and Fisheries in the Philippines since January 2002. The project has been working towards scaling-up the geographic scope to achieve the desired results in fisheries ecosystem management by expanding from a municipal up to a much broader collaboration at the inter-municipal, provincial and regional scale. It focuses on activities that address the needs in institution building and strengthening, fisheries management, habitat management, foreshore management, and coastal law enforcement. Creating marine protected area networks at adequate ecological scales do not fit into existing single municipal waters governance frameworks in the Philippines. Achieving ecosystem-based fisheries management will require developing new governance arrangements across municipalities and the cooperation of municipal leaders who recognize the need to manage fisheries at ecosystem scales.

Key words: ecosystem, governance, scaling-up, collaboration, management

Introduction

The Philippines adopts a decentralized approach to coastal management, which includes establishment and management of marine protected areas (MPAs). Each local or municipal government authority exercises management powers and possesses responsibilities over their coastal zone measured from the shoreline to 15-km seaward of municipal waters.

To improve marine habitats and enhance fishery resources, the municipal governments in Central Philippines have now established over 300 MPAs. Unfortunately, only 20-30% of these MPAs are effectively managed with sustainable fishery benefits (White et al. 2006). With the increasing number of MPAs, the need for joint management efforts in order to increase MPA management effectiveness at an ecological scale has arisen. There is likewise a need to protect areas beyond MPA boundaries in order to optimize benefits from these no-take zone areas.

At the same time, there is an increasing recognition of the need to manage no-take or restricted take MPAs at an ecosystem scale, following the ecosystem-based management approach. But the decentralized governance structure has posed a seemingly insurmountable challenge towards effective management of these MPAs.

Materials and Methods

This paper draws from the management strategies, outcomes and lessons of the Local Governance for Coastal Management Project for the Management of Coral Reefs and Fisheries in Central Philippines supported by the David and Lucile Packard Foundation since January 2002. It details the evolution of integrated coastal management practices in the municipal waters of a single municipality into an expanded management and governance arrangement by taking into consideration a fisheries

ecosystem covering multiple municipalities in Central Visayas, Philippines (Fig.1). In particular, the paper intends to show initial steps towards EBM starting from a municipal level MPAs with emphasis at scaling up to MPA networks with a facilitating inter-municipal governance structure. It captures the process of how the EBM strategy was used to address common issues that needed to be commonly addressed by a group of municipalities. Information obtained for this paper stemmed mainly from internal project reports and biophysical monitoring results of the project.

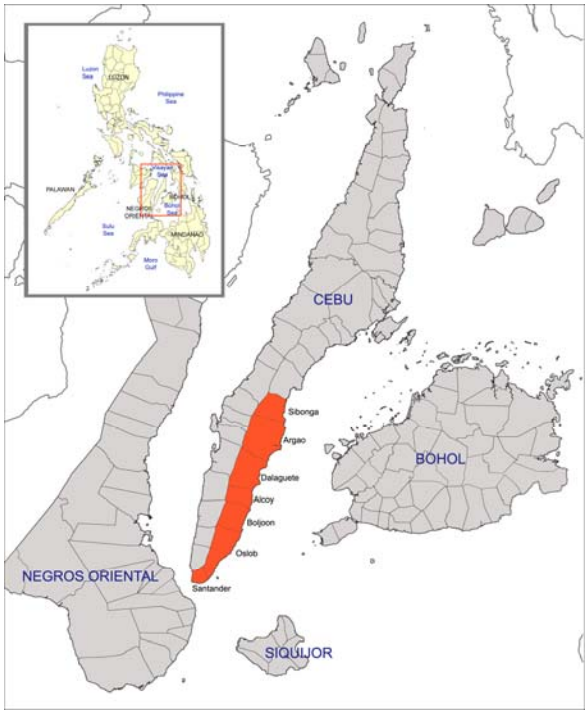

Figure 1. Southeastern Cebu municipalities, Central Visayas, Philippines

The Ecosystem Boundaries

The Philippines is considered to have coastal management programs functioning in 1/6 of 18,000 km of shoreline (White et al. 2004) with an emphasis on establishment of marine protected areas (MPAs) and fisheries management. The municipalities achieved most of the key coastal resource management (CRM) benchmarks such as:

- Adoption and implementation of a multi-year CRM plan providing overall framework and direction in managing the coastal resources of the municipality/city;
- Coastal environmental profile developed through secondary data compilation and baseline assessment (e.g., participatory coastal resource assessment, rapid assessment, scientific surveys) of coastal resources and socioeconomic and environmental conditions;
- Annual programming and budget to sustain local CRM plans and programs, with trained staff and operating municipal/city CRM unit or office; and
- CRM-related organizations in the form of People's Organizations, Fisheries and Aquatic Resource Management Councils, or Technical Working Groups, formed and active.
- Shoreline/foreshore management measures planned

The municipalities have likewise initiated various CRM best practices, such as municipal water delineation, MPAs, mangrove management, fisheries management, local legislation identified in the CRM plan, coastal law enforcement, coastal environment-friendly enterprise development, revenue generation, and multi-institutional collaboration for CRM. These benchmarks/series of tools are considered the minimum that a municipality should implement if it adopts coastal and fisheries management as a basic service (DENR 2003).

Thus far, more than 1,000 community-based MPAs (Arceo et al. 2007) have been established all over the country.

The Study Area

In Central Philippines, particularly Southeast Cebu, eight coastal municipalities traverse an irregular coastline of about 118 km. The area is bounded on the east by the Cebu/Bohol Strait, a body of water separating the islands of Cebu and Bohol with more than 158.6 km^2 of coral reef ecosystem. The eight coastal municipalities have a >125,000 ha of total combined area of municipal waters.

Based on a comprehensive study on the fisheries profile by Green et al. (2003) seven fisheries ecosystems in Central Visayas were identified. These ecosystems were considered as discrete ecosystems wherein within each ecosystem are multitudes of habitats, such as mangrove forests, coral reefs, seagrass, mudflats, sandy beaches and others. These habitats are the main feeding grounds, nursery areas and spawning grounds of coastal-dwelling marine aquatic organisms. These ecosystems, also known as the Visayan Seas, are also considered as one of the marine priority conservation areas in the Philippines. Over 400 MPAs have been established so far in the 4 Provinces of Central Visayas (UPMSI Database 2007) to improve marine habitats and enhance fishery resources, with only 20-30% of MPAs effectively managed with sustainable fishery benefits.

The Cebu/Bohol Strait fisheries ecosystem was identified as one of these fisheries ecosystem in the

Central Visayas region. It was found to contain 1,500 identified aquatic and marine species moving around areas of up to 5,000 km^2. It also supports over 5,500 small-scale fishers, however, there are existing massive threats to coral reefs and associated habitats, foremost of which is the over-exploitation of coastal and marine resources.

Despite all these obstacles, the Southeast Cebu municipalities have established 22 MPAs, thus far, in the Cebu/Bohol Strait in about 300 ha. consisting of about .24% of the total combined municipal waters.

Rationale for the EBM approach and scaling-up of MPAs

With the increasing number of MPAs established in Southeast Cebu, the need for joint MPA management efforts between the eight municipalities in order to increase management effectiveness as well as to protect areas beyond MPA boundaries has arisen. These municipalities are primarily mandated to manage their respective municipal waters under Philippine fisheries laws. Sweeping and comprehensive powers were granted through local devolution for direct management of municipal waters, hence, these powers by themselves provide inherent challenges in terms of expanded management required within ecosystem boundaries. Joint management efforts of a single ecosystem go beyond MPA and/or municipal boundaries to include multiple municipal jurisdictions.

In previous years, the said municipalities have begun implementing their CRM plans, mostly focusing on establishment of MPAs and have likewise achieved key CRM benchmarks as indicated above. It was, thus, well-placed for "scaling up" to MPA networks as ecosystem based management commonly involves "scaling-up" from design of individual protected areas to planning for MPA networks.

In Search of a definitive EBM structure

Municipal waters in the Philippines are primarily managed by municipalities. However, one of the most frequently cited reasons for weak implementation of coastal management programs through subordinate units of government at provincial and local levels is the lack of resources and capacity to carry out the required tasks. In fact, most coastal municipalities/ cities have limited financial and technical resources

To overcome these obstacles as well as to address the need for municipalities to 'scale-up' resource management and governance at a broader geographic scale, the eight coastal municipalities decided to sign a Memorandum of Agreement and create the Southeast Cebu Coastal Resource Management Council in 2005. This conforms to the current legal/institutional regime in the Philippines. Philippine laws recommend integrated management of contiguous fisheries areas.

Specifically, the State policy espoused by the Philippine Constitution provides that *"Local government units may group themselves, consolidate or coordinate their efforts, services, and resources for purposes commonly beneficial to them."* The Local Government Code of 1992 or Republic Act 7160 likewise grants genuine and meaningful local autonomy to municipalities whereby more powers, authorities and responsibilities are granted to municipalities in the management of their municipal waters. This includes the mandate for innovative and collaborative partnerships through appropriate ordinances in order to contribute funds, real estate, equipment and other kinds of property and appoint or assign personnel as may be agreed upon by the participating municipalities through a Memorandum of Agreement. In 1998, the Philippine Fisheries Code (Republic Act 8550) adopted the concept of integrated coastal management for the management of fishery and aquatic resources by municipalities and/or cities. It declares that it is the policy of the State to *"manage fishery and aquatic resources, in a manner consistent with the concept of an integrated coastal area management in specific natural fishery management areas, appropriately supported by research, technical services and guidance provided by the State."*

In the same manner, the Fisheries Code maintains that municipalities which share or border common water resources may group themselves and coordinate with each other to achieve the objectives of integrated fishery resource management in contiguous fishery resources such as bays, lakes and gulfs which straddle several municipalities.

It is clear that there are existing legal precedents that mandate municipalities to collaborate and build on existing and on-going municipal initiatives for coordinated coastal and fisheries management. Thus, MPAs are now increasingly managed collectively thru a "cluster" of municipal governments. In Southeast Cebu, there are now 22 MPAs (ave. of 13.6 ha) in about 300 ha with no-take areas covering .24% of total combined municipal waters. And these are currently managed as a MPA network by the cluster of eight municipalities.

To ensure collaboration and collective responsibility, a coordinating body for all identified programs of the eight municipalities was formed through the Southeast Cebu Coastal Resource Management Council. It is composed of Mayors and Vice-Mayors of eight municipalities that functions as a policy-making and supervising body. A complete set of officers led by a Chairman is elected among the Vice-Mayors of the municipalities who shall hold

their positions for three years co-terminus to their term of office as elected officials.

To assist the Council, a management committee and a secretariat, composed mostly of technical staff from each municipality, was organized. In addition, a technical and legal advisory group coming from government agencies such as the Department of Environment and Natural Resources, Bureau of Fisheries and Aquatic Resources, and Philippine National Police, as well as NGOs such as the Environmental Legal Assistance Center and the CCEF, provides specialized support to the Council. The Council often calls on any of these government agencies and/or non-government organizations for technical inputs, legal advice, logistical support, and other forms of technical assistance. The management committee headed by an Executive Director administers the day-to-day activities of the Council while the secretariat serves as the record keeper and financial manager. Both the management committee and secretariat have provided an effective coordination and feedback mechanism to respective municipalities.

Significant Socio-Institutional and Bio-physical Outcomes

The Council, although still young and evolving, is now a venue for the discussion and resolution of important issues and activities. It focuses on activities that address the needs in institutional building and strengthening, fisheries management, habitat management, foreshore management, and coastal law enforcement.

Through the Council meetings, issues such as the banning of compressor fishing; protection of critical habitats from foreshore development; the reduction of fishing effort through fisherfolk, fishing gears and boat registration, regulating *pa-aling* (modified *muro-ami*) and banning *sagiwsiw* (modified drift gill net) fishing, and strict enforcement against intrusion of commercial fishing into municipal waters. The Council has likewise considered other concerns such as the oil seismic survey along Cebu Strait; regulating municipal tourism activities; and creating social networks among MPA managers. In effect, the multi-municipality MPA network has developed into a strong institution capable of effective coordination and management actions.

It has also successfully opened a venue for the provincial government to downstream support for coastal management at the inter-municipal levels. Some contextual issues such as weak formal institutions and three-year terms of municipal mayors (Christie, et al 2007) have been avoided as well. Periodic political and leadership changes have not affected coastal management programs with the smooth transition, stability, and continuity of the Council and its functional committees.

Through the Council, municipal coastal management initiatives in the eight municipalities have been re-energized. MPAs are now effectively managed, with initial results from biophysical monitoring suggesting an increasing trend in percentage of live hard coral cover recorded inside MPAs (Figure 2) and fish abundance of commercially valuable fishes (target fishes) (Fig.3).

Biophysical reef monitoring of MPAs involves a participatory method in estimating fish populations and substrate composition with competent local community members. Surveys are conducted in the shallow reef (3-4m depth) and deeper portion of the reef (7-9m depth) both inside and outside the MPA boundaries for comparative changes due to protection.

The results of the monitoring surveys have been used as inputs for MPA management decision-making at the municipal government and municipal cluster levels.

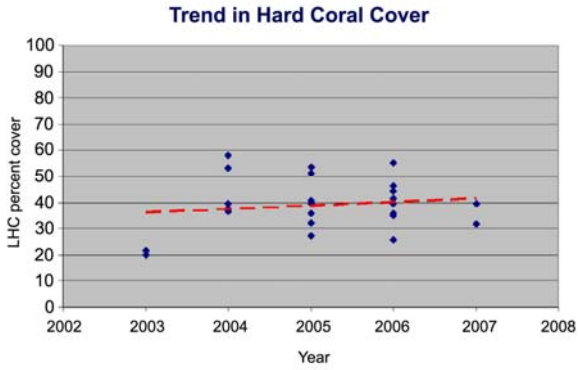

Figure 2. Trend in Hard Coral Cover of SE Cebu MPAs

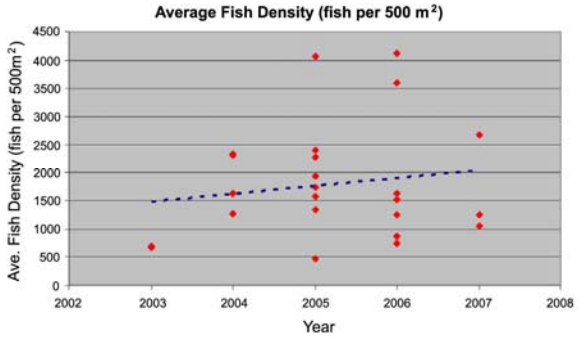

Figure 3. Trend in Average Fish Density of SE Cebu MPAs.

With improvements in reef status, management effectiveness of reef managers has likewise increased by at least one degree using the MPA Rating and Database System developed by the Coastal Conservation and Education Foundation, Inc. It is a

point-based rating system that is designed to aid MPA managers in evaluating their performance in terms of management, implementation, and enforcement (White et al. 2006). The extent of management is measured according to: enforcement, education, capacity development, biophysical monitoring, and sustainable financing, with Level I (MPA is initiated) up to Level V (MPA is institutionalized.

There has also been a significant reduction of threats to the coastal habitats through improved efficiency and cost-effectiveness of joint management actions (e.g., law enforcement). Municipal seaborne and coastal law enforcers (MUSCLE) have been formally organized with an institutionalized incentive scheme. To supplement the monitoring, control and surveillance of the MUSCLE, joint seaborne operations have also been supported by the Council. Currently, over 30 commercial fishing violators have been apprehended during joint law enforcement operations since April 2005. With the success of the SE Cebu cluster, other municipalities in the Province are now doing corresponding processes of clustering into inter-municipal governance arrangements.

Lessons: What will determine the right fit?

It is important to adjust natural resource governance regimes to the size and nature of the ecosystem, and to work within the range of administrative arrangements allowable to find the right fit. The administrative boundaries that fit the demands of EBM will vary according to the local context. In the Philippines, the right ecosystem fit depends on the manageability and consensus among leaders of municipal government units. It cannot be a "one ecosystem size for all" approach in EBM in the Philippines. What size is appropriate will depend on the nature of the resource(s) to be managed and the drivers that determine the status of the resource on the one hand, and the mutual decisions of the leaders to work together to manage these environmental, social and economic drivers that are affecting the system—in this case the fisheries ecosystem.

The extent of the Cebu/Bohol Strait fisheries ecosystem is limited to the municipal water boundaries of 8 municipalities. A separate management structure was organized by these municipalities in order to provide a platform for ecosystem-based management of this fisheries ecosystem. This inter-municipal management council – known as the Southeast Cebu Coastal Resource Management Council – has become the commonly-agreed upon and committed governing body for "scaling-up" to EBM of the network of MPAs found therein, and hopefully the marine space in-between.

Conclusion

Spatial scales for ecological marine protected area networks do not fit into existing single municipal waters governance framework in the Philippines. Thus, to be effective in managing ecosystem boundaries, there is a need to expand and create a separate governing authority to govern multiple municipal governments. With ecosystem values foremost in the management considerations, governance regimes in the Philippines allows for such expansion or "scaling up." Inevitably, ecosystem boundaries are usually set using a balance of ecological functions and appropriate governance considerations.

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The author would like to express her gratitude to Dr. Alan T. White and Dr. Patrick Christie who provided helpful insights to some portions of this paper. This work would not have been possible without the support of the Coastal Conservation and Education Foundation (CCEF), Inc. with financial assistance from the David and Lucile Packard Foundation. Finally, I would like to express my sincerest thanks to the hardworking men and women of the CCEF, especially Rizaller Amolo, Sheryll Tesch and Aileen Maypa.

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Post hurricane dynamics and status of coral reefs St. Croix, US Virgin Islands

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Abstract. To protect the vitality of Caribbean reefs, it is important to understand the physical and biological factors affecting the status of corals. Hurricane Hugo caused extensive damage on the island of St. Croix, US Virgin Islands in 1989. Underwater transects were set up on Tague forereef in 1989 and 2007 to study the coral species. The data show that there was an increase in both overall live coral species richness and total live coral present on Tague forereef eighteen years after Hurricane Hugo. These data were also compared with those collected on the south forereef of Buck Island Reef National Monument to assess the state of a protected reef after the hurricane. No significant difference was found between the percentage of live coral cover on Buck Island south forereef and Tague forereef in 2007. This suggests that marine protected areas alone cannot prevent the degradation of Caribbean coral reefs, and more effort must be placed on large-scale prevention of tidally transported coral-damaging factors.

Key Words: Caribbean, Hurricane, Marine Protected Area

Introduction

Hurricanes are acute physical factors that have immediate effects on the dynamics of coral reefs (Bythell et al. 1993b; WS1 2008). The intensity of hurricanes is escalating (Lugo 2000; Aronson and Precht 2006; WS1 2008)—under doubled CO_2 conditions they are estimated to be 10-20% stronger (Kleypas et al. 2001). Strong currents, large tidal ranges and transported debris can knock over large branches, roll heads of coral and smother live coral polyps (Bythell et al. 2000). According to the Intermediate Disturbance Hypothesis (Connell 1978) hurricanes can have positive effects—when of intermediate frequency—on coral reefs by clearing suitable substrata for new coral recruits that might have otherwise been outcompeted for space (Bythell et al. 2000). However, the analysis of the long-term consequences of major physical effects of storms is complicated by chronic biological factors (Bythell et al. 1993a; Bythell et al. 1993b; Bythell et al. 2000; Humann and DeLoach 2002; Aronson et al. 2005; WS1 2008).

On September 17-18, 1989, Hurricane Hugo raged over St. Croix for more than 12 hours (Hubbard et al. 1991; Bythell et al. 1993a). The category 4 storm was the first hurricane since 1928 to directly hit the island of St. Croix (Hubbard et al. 1991; Bythell et al. 1993a; Bythell et al. 2000). The near-shore reefs on St. Croix endured large waves, estimated at 6-7 m in height on Tague Reef, which caused erosion and increased sediment transfer (Hubbard et al. 1991). This study aims to analyze the effects of a major disturbance on the reefs of St. Croix.

Caribbean coral reefs have undergone a phase shift from coral to algal dominance since the late 1970s (Aronson and Precht 1997; Graham et al. 2006). These shifts have primarily been attributed to over-fishing as well as the Caribbean-wide disease related die-off of the echinoid, *Diadema antillarum* in 1983-1984 (Humann and DeLoach 2002; Aronson and Precht 2006; Mumby et al. 2006). Without the presence of the dominant algal grazer, *D. antillarum,* on Caribbean reefs, herbivorous fish such as parrotfish and surgeonfish, have become the dominant macroalgal grazers. Most marine protected areas (MPAs), such as Buck Island Reef National Monument (BIRNM), are primarily established to protect over-fished populations of tropical fish (Hughes et al. 2002). By restoring the abundance of herbivorous fish, MPAs are proposed to protect corals by increasing the grazing pressures on the macroalgal communities thus allowing colonization space for coral recruits (see Aronson and Precht 2006 for a review of related studies). Comparison of data from protected and unprotected reef sites on St. Croix suggests that the protection of a reef alone does not allow for a higher colonization of hard corals.

Materials and Methods

St. Croix Island is located in the Caribbean Sea and is a part of the USVI (Fig. 1A). Tague forereef

protects the northeast shore of the mainland in an east-west orientation.

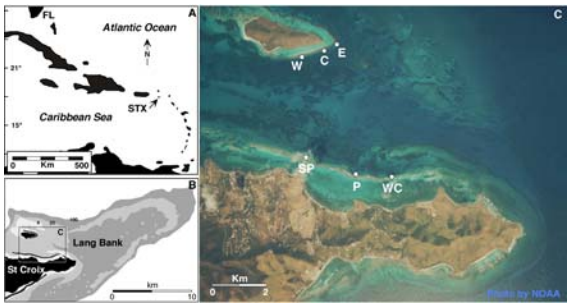

Figure 1: Map showing study localities: A) Island of St. Croix . B) Buck Island Reef National Monument and Tague Reef. C) Three study sites on Tague Reef and Buck Island south forereef. Tague Reef localities are Whaler Cut (WC); Platform (P); and Solar Panel (SP). Sites on Buck Island south forereef are West (W), Center (C), and East (E).

BIRNM is located 2.5 km off the northeast coast of St. Croix. Established as a National Monument in 1961, the continuous barrier reef forms an arc on the north and south-east side and protects the island from a primarily easterly wave action (Hubbard 1991). The southern forereef (Fig. 1C) has been monitored since 1988. The south forereef is usually protected from 'near-miss' hurricanes due to its proximity to the mainland of St. Croix, but when Hurricane Hugo passed directly over the island, BIRNM south forereef was hit harder than any other on St. Croix (Hubbard et al. 1991; Bythell et al. 1993a).

In the winter of 1989-1990, about three months after Hurricane Hugo struck St. Croix, data were collected at three locations on Tague forereef (Fig 1C) (Hubbard 1991; Hubbard et al. 1991; Hubbard pers. comm. 2007).

New data collections during the summer of 2007 were performed at the same three locations on Tague forereef—located by GPS—and at an additional three locations on Buck Island south forereef (Fig. 1C) (Fisco 2008). A meter tape was used to measure every contour and type of substrate—live coral, dead coral, coral rubble, sand and pavement—along the horizontal chain transects. The species of live coral, dead coral and coral rubble was recorded.

An assessment of the total live coral of each species was performed between 1989 and 2007, and differences in species richness and dominant species were noted. The species composition and total percent live coral cover of the substrate were compared between Buck Island south forereef and Tague forereef to assess the differences between a protected reef and one that is not.

Results

The results of this research are of two kinds. The first set of data analyzes the trends in species richness at the three Tague forereef locations between 1989 and 2007. This includes an analysis of low relief vs. high relief species. Low relief species are smaller species of coral which form encrusting colonies, up to 0.6 m in diameter, and therefore are not significant reef builders (Humann and DeLoach 2002). High relief species are those which grow to be as big as 4 m in diameter and add significantly to the structure of the reef (Humann and DeLoach 2002). The second set of data shows trends in total live coral cover between 1989 and 2007 at the three Tague forereef locations as well as a comparison of live coral between Tague forereef and Buck Island south forereef.

Trends in Species Richness

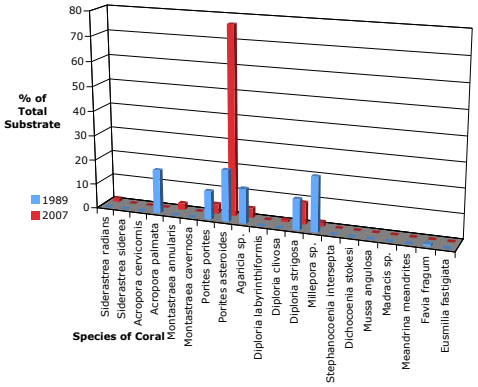

Figure 2: Comparison graph showing the percentage of total live coral of each species between 1989 and 2007 at 3 m depth, averaged between the three locations: Solar Panel, Whaler Cut and Platform.

The 3 m depth transects did not display a large species number influx in the eighteen years between Hurricane Hugo and the 2007 survey. On average, only one more species was found at the 3 m depth (Fig. 2). *Favia fragum* died out completely, as did *Acropora palmata*. *Siderastrea radians, Montastraea annularis,* and *Diploria clivosa* are three smaller, low-relief coral species that were not represented in the 3 m assemblages in 1989 but were present on the reef in 2007.

With a total of four species, the number of species of corals at 6 m was relatively low in 1989, this number more than doubled by 2007 with thirteen species present in the three locations (Fig. 3). In both 1989 and 2007, *Porites porites* represents the highest proportion of live coral present at 6 m on Tague forereef. In 2007, the low relief species *Agaricia* sp. (22.5%), *Siderastrea radians* (15.7%) and *P. asteroides* (15.4%) were

relatively dominant. All of the species present at the 6 m depths are considered low relief, non-framework building corals. Although no species had died out, eight new species had colonized the 6 m depth of Tague forereef by 2007.

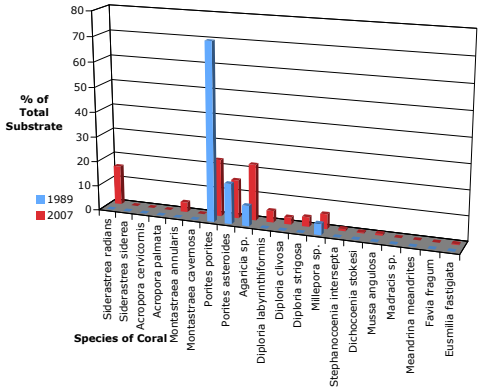

Figure 3: Comparison graph showing the percentage of total live coral of each species between 1989 and 2007 at 6 m depth, averaged between the three locations: Solar Panel, Whaler Cut and Platform.

The 9 m depth also showed an overall trend of increasing species richness. While only four species were recorded in 1989, the 9 m depth experienced a threefold jump in number of species to twelve species in 2007 (Fig. 4). In one of the transects, Whaler Cut 6 m, 100% of the live coral assemblage was composed of *M. annularis* in 1989. In 2007, at this same transect, the *M. annularis* population accounted for only 8.3%, while *Porites porites*, a smaller branching coral, dominated the assemblage with 32.2% of the live coral cover.

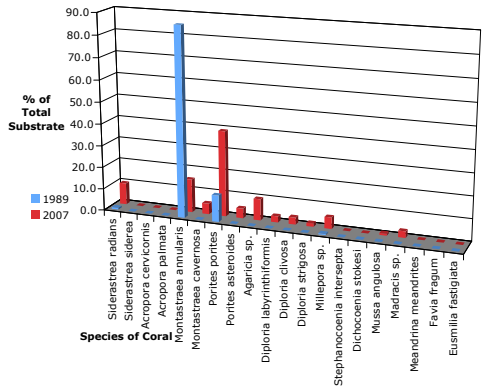

Figure 4: Comparison graph showing the percentage of total live coral of each species between 1989 and 2007 at 9 m depth, averaged between the three locations: Solar Panel, Whaler Cut and Platform

Trends in Live Coral Cover

Table 1: Total percent of the reef composed of live coral cover in 1989 and 2007.

Year	Total % Live Coral
1989	10.5%
2007	12.3%

On average, the total amount of live coral present on Tague forereef increased by 2.3% in the eighteen years after Hurricane Hugo (Table 1). Although an increase was observed, the percent of live coral present on the reefs in both studies was very low.

Table 2: Percent live coral cover of total substrate by location and depth on Tague forereef. Depths that experienced an increase in coral cover over the 18-year period are underlined.

Depth	Year	Location		
		Solar Panel	Whaler Cut	Platform
3 m	1989	6.6%	2.6%	3.2%
	2007	*22.3%*	*6.2%*	*7.7%*
6 m	1989	9.0%	26.8%	10.9%
	2007	*16.1%*	9.0%	7.2%
9 m	1989	0.0%	18.4%	17.3%
	2007	*1.9%*	7.6%	9.1%
Average	1989	5.2%	15.9%	10.5%
	2007	*13.4%*	7.6%	8.0%

Though there was a general trend towards increasing species richness in all but one transect between 1989 and 2007 (Fig. 2-Fig. 4), the Whaler Cut and Platform transects showed a decrease in the average percent of live coral cover (Table 2). The Whaler Cut 6 m transect showed the greatest change in coral cover—a 16.8% *decrease* in live coral cover occurred between 1989 and 2007. All of the depths at the Solar Panel transect showed a relatively large increase (an average increase of 8.2%) in live coral.

Table 3: Average total live coral measured between Buck Island south forereef and Tague forereef in 2007. The percent of the substrate covered by live coral cover is listed in parentheses.

	Average total live coral measured (m)	
Depth	**Buck Island**	**Tague Reef**
3 m	2.15 (15.7%)	1.60 (14.2%)
6 m	1.71 (10.9%)	1.64 (12.9%)
9 m	1.67 (10.4%)	1.21 (9.8%)
Average	1.84 (12.3%)	1.48 (12.3%)

In 2007, there is almost no difference (0.36 m) between the total amount of live coral present on Tague forereef and Buck Island south forereef. The average percent of the substrate covered by live

coral is the same on Buck Island National Monument and Tague forereef (Table 3).

The coral species present at each reef are different but on the whole, the reefs of St. Croix and Buck Island south forereef contain relatively small, non-framework building corals.

Discussion

Because of the close interaction between humans and the natural environment, it is important to understand how ecosystems react and respond to major disturbances such as hurricanes so that we can try to adjust and plan accordingly.

In a paper detailing the effects of hurricanes in the Caribbean, Lugo (2000) discusses the impacts on terrestrial vegetation. The pattern witnessed by Lugo (2000) closely resembles that of the Intermediate Disturbance Theory proposed by Connell (1978). At first, in terrestrial environments, there is massive mortality of vegetation over a short time period (Lugo 2000; Done, 1999). This mass mortality is followed by a delayed pattern of tree mortality (Lugo 2000). The final step is a high turnover of terrestrial species.

The reefs of St. Croix post-Hurricane Hugo are a good example of Lugo (2000) and Connell (1978)'s models. At first, Bythell et al. (1993a) recorded a loss of 40-46% of the pre-hurricane live coral cover on Buck Island south forereef. Hubbard et al. (1991) also reported near total destruction of the south facing reefs on Buck Island. Next, Bythell et al. (2000) found that the coral abundance on the reefs of St. Croix remained in the immediate post-hurricane condition for two to three years before returning to approximate pre-hurricane levels by June 1991 (Bythell et al. 1993a).

Lugo's last observation is clearly demonstrated, as there were only two locations at which the dominant coral species did not change in the eighteen years between the two studies on Tague forereef, and species richness increased in all but one transect. Bythell et al. (2000) found that the recruitment of coral species on the south side of BIRNM was strong following Hurricane Hugo. The massive sediment transfer, reported by Hubbard et al. (1991) on the reefs of St. Croix could allow for settlement 'colonization gaps' in which new recruits with better regeneration strategies could settle and flourish. Bythell et al. (2000) found that the area most severely impacted by hurricane disturbance demonstrated the greatest increase in species richness of all the sites studied due to high recruitment of uncommon species into the newly cleared niches. These high recruitment rates are essential to sustaining the ability of the reefs to re-establish complex communities in the wake of hurricane damage (Bythell at al. 2000).

An apparent shift has occurred from high relief, framework building corals to smaller, low relief, non-framework building corals, soft corals and macroalgae (Aronson and Precht 1997; Done 1999; Aronson and Precht 2006). Population modeling suggests that an increase in the frequency and/or the intensity of disturbances should tend to skew size frequency distributions towards smaller size classes of organisms (Done 1999). These population models could help explain why there was a large increase in dominance of small, low relief coral populations on the reefs of St. Croix.

Reef building by larger corals is very important for reefs to cope with global sea level change (Grigg 1998; Kleypas et al. 2001). Globally, there are many documented cases where reefs have failed to keep up with Holocene sea-level rise and subsequently contain no live corals (Kleypas et al. 2001). The rate of reef growth in these cases was too slow to keep the corals and their algal symbionts in the photic zone. The production of calcium carbonate by larger, high relief, branching corals also provides topographical complexity, including nooks and crannies that support the vast number of reef species.

BIRNM has been nationally protected for 46 years, but no significant difference was found between the percent live coral cover on Buck Island south forereef and Tague forereef (Table 4). In a study conducted concurrently with this study, and at the same locations on Tague forereef and Buck Island south forereef, Burpee (2007, pers. comm.) found that the ban on fishing established on BIRNM has been successful in protecting the herbivorous and predatory fish populations. Overall, he found that the parrotfish on Buck Island south forereef are larger and in higher abundance than those on Tague forereef. Common sense and many scientific studies lead to the conclusion that the increase in size and number of grazers would produce a decrease in macroalgal cover and provide suitable settlement substrata for new corals (Mumby et al. 2006; see also Aronson and Precht 2006 for a review of related studies). As Table 4 shows, however, there was no significant difference between the live coral cover on Tague forereef and Buck Island south forereef.

These results reveal that local conservation efforts of MPAs are only a first step in the conservation of coral reefs (Reaser et al. 2000; Hughes et al. 2002). There are many Caribbean-wide factors, such as diseases and climate change, affecting coral reefs that cannot be prevented by eliminating fishing pressures in an MPA (Aronson

and Precht 2006). MPAs can, however, be greatly aided by better management of surrounding ecosystems and more effective, stronger international efforts to reduce global warming (Reaser et al. 2000; Hughes et al. 2002; Aronson and Precht 2006).

The extent of damage from tropical storm and the subsequent recovery of reefs are highly variable between reef sites (Bythell et al. 1993a), and we are just beginning to understand influences on coral reef community structure (Bythell et al. 1993a). The data collected in 1989 (Hubbard 2007, pers. comm.) and 2007 should help contribute to future analyses of the effects of tropical storms on the reefs of St. Croix. More frequent and regular surveys should be performed on St. Croix and Buck Island reefs to evaluate exactly how the communities adapt to large-scale disturbances.

Conclusions

Coral reef communities are dynamic and are constantly driven to change due to many biological and ecological processes, including environmental disturbances (Aronson and Precht 1997). Overall the live coral species richness has increased on Tague forereef, St. Croix, in the eighteen years since Hurricane Hugo. There were no noticeable differences in live coral cover between the protected reef on Buck Island Reef National Monument and that on the main island of St. Croix. This research shows that annual surveys of MPAs will be crucial for determining the role they can play in maintaining and/or protecting enough live coral to help re-populate reefs in the wake of ecosystem change. Without efforts to tackle global environmental issues, MPAs will not fix the problem of worldwide coral decline (Aronson and Precht, 2006).

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Water quality standards for coral reef protection

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Abstract. The U.S. Clean Water Act provides a legal framework to protect coastal biological resources such as coral reefs, mangrove forests, and seagrass meadows from the damaging effects of human activities. Even though many resources are protected under this authority, water quality standards have not been effectively applied to coral reefs. The Environmental Protection Agency is promoting biocriteria and other water quality standards through collaborative development of bioassessment procedures, indicators and monitoring strategies. To support regulatory action, bioassessment indicators must be biologically meaningful, relevant to management, responsive to human disturbance, and relatively immune to natural variability. A rapid bioassessment protocol for reef-building stony corals was developed and tested for regulatory applicability. Preliminary testing in the Florida Keys found indicators had sufficient precision and provided information relevant to coral reef management. Sensitivity to human disturbance was demonstrated in the U.S. Virgin Islands for five of eight indicators tested. Once established, monitoring programs using these indicators can provide valuable, long-term records of coral condition and regulatory compliance.

Key words: Clean Water Act, biocriteria, coral reefs, water quality standards

Introduction

Coral reefs worldwide suffer adverse effects from human activities (Lough, 2008; Knowlton and Jackson, 2008; Mora, 2008; Sandin et al. 2008). Marine Protected Areas (MPAs) are widely used in coral reef conservation to restrict fishing, diving and boating in selected areas, but cannot protect coral reefs from the many other human activities that degrade coastal zones from upstream watersheds (pollutant discharge, urban and industrial activity, agriculture) and outside MPA boundaries (dredging, dumping, fishing).

Fortunately, the Clean Water Act (CWA) provides a legal and regulatory framework to protect aquatic resources, including coral reefs, mangrove forests, wetlands and seagrasses in U.S. territorial waters. The CWA mandates that states and territories protect and restore the chemical, physical, and biological integrity of the Nation's waters (CWA Section 101 (a)). Protection extends to all territorial waters within three miles of shore, not just those within MPA boundaries, and regulatory responses to impairment can influence a range of management actions and decisions in both the coastal zone and watershed (e.g., fishery restrictions, land use allocations, building permits, effluent discharge permits). This comprehensive scope makes the CWA a powerful tool, having the potential to integrate conservation efforts across municipalities, coastal zones and landscapes. Moreover, designated waterbody uses (what to protect) and water quality standards (the level of protection) are state decisions, and therefore represent regional cultural and socioeconomic values.

The U.S. Environmental Protection Agency (EPA) is promoting development and implementation of coral reef biological criteria (biocriteria) as the most direct application of the CWA for protection and restoration of coral reefs (see Bradley et al. 2008a). The CWA requires that States and Territories define water quality standards for all waters. Water quality standards include 1) designated uses reflecting societal goals for the water body, 2) criteria to achieve the designated uses, and 3) antidegradation policies to prevent deterioration of high-quality waters. Criteria define the thresholds

(expectations) for biological, chemical, or physical indicators of resource condition and may be described in narrative or numeric terms. When biological criteria are adopted, they are legally binding—carrying both an obligation and authority to maintain the specified biological integrity. If a waterbody fails to meet the criteria, then it fails to support the designated uses and is listed as impaired in the CWA Section 303(d) report to Congress. Cause of impairment must be determined and restorative measures initiated.

Biocriteria have been broadly applied in the protection of streams, lakes, and estuaries (EPA 2002), but not coral reefs and other nearshore habitats. An important advantage of biological over physical and chemical water quality standards is that living organisms integrate the effects of multiple stressors through time, providing a reliable indicator of ecological condition (Jameson et al. 2001) rather than independent snapshots of single stressors. Biological endpoints are also transparent to stakeholders and can guide and support management decisions. Moreover, bioassessments and biological endpoints are directly responsive to the fundamental purpose of the CWA—protection of valued aquatic resources. Bioassessments incorporated into a long-term monitoring program could simultaneously provide diagnostic, management and regulatory information.

The lack of coral reef biocriteria in U.S. jurisdictions prompted EPA to initiate a collaborative program to foster development of water quality standards specifically to protect coral reef communities (Bradley et al. 2008b). A first objective was to develop bioassessment indicators and protocols suitable for regulatory application. Legally defensible monitoring programs and indicators must be scientifically sound, biologically meaningful, relevant to management, predictably responsive to human disturbance and relatively immune to natural variability. These factors are examined here for a recently introduced assessment protocol for stony corals.

The *Stony Coral Rapid Bioassessment Protocol* (RBP; Fisher 2007) was developed to capture both colony and surface area attributes of stony corals. Stony corals directly provide valuable ecosystem services (e.g., shoreline protection, community habitat) and form the infrastructure of most coral reefs. The potential of the protocol for development of biocriteria was examined in studies performed in the Florida Keys and U.S. Virgin Islands.

Material and Methods

The RBP consists of three simple underwater measurements; coral identification, coral size (height, diameter and width), and percent living tissue (Fisher 2007). Because most colonies were hemispheric, the average radius of each colony was converted to three-dimensional colony surface area (SA) using a hemispheric surrogate ($SA=2\pi r^2$). Colonies of the most abundant species in Florida and the Caribbean are hemisphere or dome-shaped, and this surrogate was found to estimate surface areas within 17% of actual (Courtney et al. 2007). Different coral species would require different geometric surrogates. Indicators (Table 1) included taxa richness, colony density and calculations of average colony surface area, total surface area (TSA), average % live tissue, live surface area (LSA), % live surface area (LSA/TSA*100) and the coefficient of variation for average colony surface area (CSA-CV) Indicators reflect both surface area (e.g., coral cover) and colony characteristics (e.g., density, size) of corals.

Two pilot studies were performed in the Florida Keys to assess the capacity of RBP indicators to detect spatial change and to evaluate the potential utility for management decisions. Studies at St. Croix, U.S. Virgin Islands further examined the potential of RBP indicators to differentiate human disturbance from natural variability. A survey was performed at stations located across a strong human disturbance gradient (industrial ship channel) on the southern shore of St. Croix. Indicators were tested for correlation with distance away from the center of the human disturbance.

Indicators of Physical and Biological Condition

Colony density: *number of colonies per m^2 sea floor*

Species (taxa) richness:*# species occurring in a defined region*

Colony surface area (CSA): *surface area (3D) of an entire colony*

Total surface area (TSA) = *Σ CSA (m^2)*

Average colony surface area (AvCSA) = *TSA/ #colonies (m^2)*

Colony size coefficient of variation (CSA-CV)=*standard deviation CSA/mean CSA*

3D Total Coral Cover (TC) = *TSA/ m^2 sea floor*

Percent Live Tissue (%LT): *percent live tissue on a coral colony*

Average Percent Live Tissue (Av%LT) = *Σ %LT/ #colonies*

[2]**Colony Live Surface Area**: *surface area (3D) of live coral tissue on an entire colony (m^2)*

Live surface area (LSA) = *Σ colony live surface areas (m^2)*

3D Live Coral Cover (LC) = *LSA/ m^2 sea floor*

Percent Live Surface Area (%LSA) = *[LSA/TSA]*100*

Table 1: Selected indicators from observations and calculations of stony corals using the Stony Coral Rapid Bioassessment Protocol (Fisher 2007).

Results

In all three studies, indicators detected differences between stations and were determined to have sufficient

precision for most monitoring programs (Fore et al. 2006). In addition, five of eight indicators tested across a human disturbance gradient at St. Croix were positively correlated with distance from the center of the disturbance zone (for example, see Fig. 1). Indicators that were responsive to human disturbance were taxon richness, AvCSA, CSA-CV, TSA and LSA. Coral density tracked distance from disturbance but the correlation was not significant. Because of small colonies with high %LT in the disturbance zone, associations of average %LT and %LSA were negative (Fisher et al. 2008).

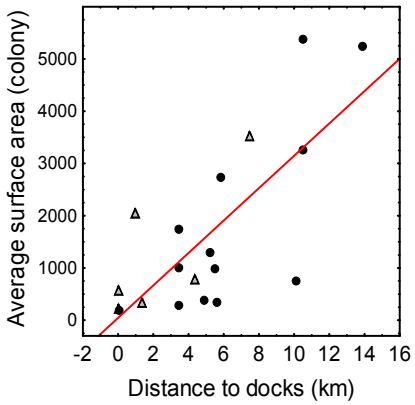

Figure 1. Relationship of colony surface area (AvCSA) with distance from commercial docks on the south side of St. Croix, USVI (from Fig. 2 of Fisher et al. 2008). This is one of five indicators that showed a predictable response from human disturbance. Triangles represent stations west of the commercial docks, circles are stations east of the docks (n = 19 stations).

In each survey, small colonies were more abundant than large colonies, but the greatest LSA was provided by mid-sized colonies (e.g., Fig. 2). Percent live tissue (%LT) declined with colony size for all species, but in the Florida Keys dropped dramatically for medium and large Elkhorn (*Acropora. palmata*) colonies (Fig. 3). Elkhorn corals provided the most reef habitat (TSA) at the Florida Keys stations, but exhibited the poorest health (LSA/TSA) relative to other species (Fig. 4). This condition was most conspicuous at a single reef, Sand Key, where numerous large *A. palmata* were devoid of live tissue (data not shown). Population structure (colony size-frequency) varied across regions for different species (Fore et al. 2006), and this was particularly evident for species at St. Croix where strong recruitment (abundant small colonies) was found at some locations and not at others (e.g., Fig. 5).

Discussion

Indicators generated by the *Stony Coral Rapid Bioassessment Protocol* (RBP) should be considered further as potential metrics in a biocriteria program. The procedures are straight-forward, inexpensive and easily measured by coral reef biologists; the indicators demonstrated sufficient statistical power to monitor for trend and detected a consistent and logical response to human disturbance over natural variability. Further, the indicators are biologically meaningful, easily interpreted by non-scientists and provide information relevant to resource management.

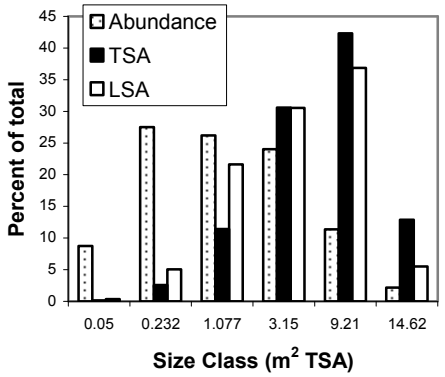

Figure 2. Relative size-class distribution of abundance (stippled bar), total surface area (TSA; dark bar) and live surface area (LSA; white bar) for *Acropora palmata* found at stations in the Florida Keys.

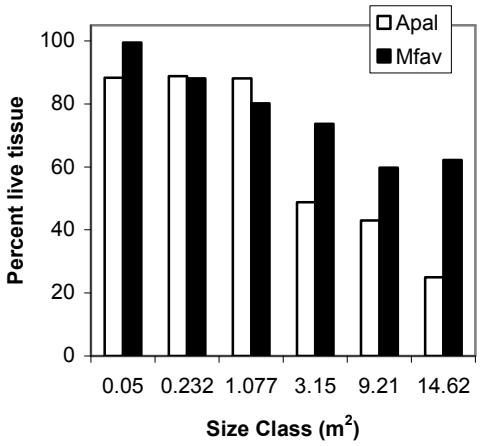

Figure 3. Comparison of the proportion of live tissue on *Montastraea faveolata* (Mfav) colonies and *Acropora palmata* (Apal) colonies relative to the size of the colonies. Large *A. palmata* had lower %LT than large *M. faveolata*.

A significant requirement for a regulatory bioindicator is predictable responses to human disturbance. It is expected that corals respond to chronically poor environmental conditions with decreasing taxon richness, size and health. In the St. Croix study, several indicators for these attributes declined consistently with distance from the zone of human disturbance. This pattern was recorded at different depths and reef habitats, signifying that the indicators were relatively

immune to changes in microhabitat (Fisher et al. 2008). Although this may not always be the case, robust indicators can overcome the need for classification (sorting reefs by habitat type) which simplifies survey designs and reduces the number of stations that must be surveyed.

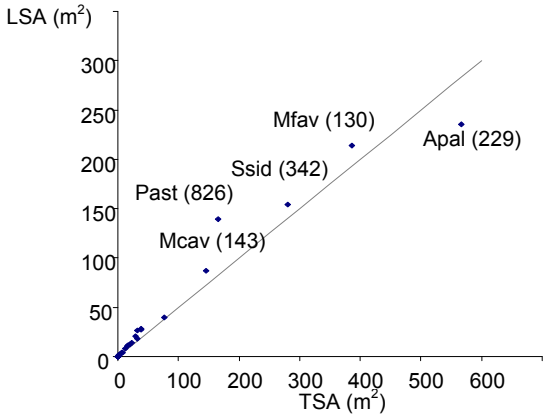

Figure 4. Comparison among coral species of live surface area (LSA; m^2) and total surface area (TSA; m^2) recorded for all colonies found at 29 stations in the Florida Keys (Mcav-*Montastraea cavernosa*; Past-*Porites astreoides*; Ssid-*Siderastrea siderea*; Mfav-*M. faveolata*; Apal-*Acropora palmata*. Colony numbers for each species are in parentheses and the dotted line represents 50%LSA.

Resource managers often focus on the condition of stony corals because they form the infrastructure of the reef and directly provide ecosystem services (e.g., shoreline protection, biological diversity, fishery harvests, and tourism). Stony corals are therefore appropriate for regulatory bioassessment. Stony coral condition has been quantified many different ways, but the RBP is unique in providing both colony and surface area data—each bearing a different subset of relevant management information. For example, the RBP includes measurements to estimate colony size, providing information on reef structural complexity, population structure and even historical condition. Likewise, the RBP includes estimates of live and total surface area, which can be used to characterize colony health and potential for growth and reproduction. The two approaches can even be combined to illustrate otherwise undocumented characteristics, such as size-related health (Fig. 3).

Colony size is sometimes ignored in coral monitoring programs, even though size provides highly relevant management information. For example, size can be used to demonstrate the size classes and species with the greatest physical capacity for growth and reproduction. In an example from the Florida Keys survey (Fig. 2), medium-sized colonies had higher LSA than small or large colonies. Areas with medium sized colonies might be expected to recover more quickly (resilience) from a bleaching event. A region with large colonies (even large, dead colonies) indicates that historic environmental conditions were capable of supporting vigorous or prolonged coral growth. A region with small colonies and low abundance may have experienced more persistent, chronic stresses. Presence of large colonies with low proportions of live tissue could indicate a region with historically good environmental conditions that suffered some catastrophic event, such as a hurricane or massive bleaching. A likely example of this was found for *Acropora palmata* at Florida Keys, a situation that was previously noted by Fisher et al. (2007). Large *A. palmata* colonies exhibited much lower %LT than large colonies of other coral species (such as *Montastraea faveolata*; Fig. 3), indicating differential sensitivity to the stressor. Smaller colonies exhibited high %LT, indicating a return to pre-event conditions for newly recruited colonies.

The plight of *A. palmata* colonies in Florida Keys was also evident—on average, colonies had less than 50% live surface area (LSA/TSA; Fig. 4). This is particularly important for resource management, not only because *A. palmata* are listed as threatened species, but also because they likely contribute more than any other species to shoreline protection, community habitat, biodiversity and tourism. Other species (e.g., *M. faveolata*) have better health but with lower TSA do not contribute as many services. Further, *A. palmata* may be critical to reef sustainability. Done (1997) claims that reef sustainability is driven by two key variables, reef framework and reef-building capacity. Elkhorn corals have already lost reef-building capacity (%LSA) and, with eventual deterioration, will contribute less and less to reef framework (TSA).

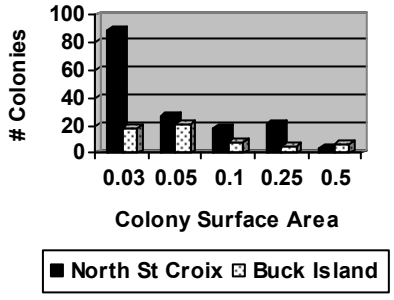

Figure 5. Number of colonies by size class (CSA; m^2) for *Diploria strigosa* from the northern shore of St. Croix (U.S. Virgin Islands) and nearby Buck Island.

Colony size can also used to compare population structure (size-frequency), which identifies populations that have high and low relative recruitment (Fig. 5). Study of population structure over time provides

essential information on population dynamics (Bak and Meesters 1998). Colony size (AvCSA) and colony size heterogeneity (CSA-CV) were among those indicators responsive to human disturbance—both were lower for stations near the center of disturbance (Fisher et al. 2008; Fig. 5). Chronic human disturbance (e.g., pollution, physical damage, overfishing) may not always inhibit coral recruitment, but poor environmental conditions will likely lead to reduced growth rates and shorter life spans.

The RBP indicators exhibited significant potential for monitoring programs, regulatory standards and management utility. Although application to other locations and environments is needed, these studies complete a first step toward development of tools for coral reef biocriteria.

Since the 1980s, there has been growing recognition that water quality standards based on chemical criteria alone cannot protect biological resources (Karr 1991). This has stimulated efforts to develop biological assessment methods and to implement biocriteria. Many states and territories now fulfill the regulatory requirements of the CWA to document the condition of streams, lakes, and estuaries through local and even regional assessments of fish, macroinvertebrates, and algae (EPA 2002). Similar programs for coral reefs can be developed and implemented to provide useful management information and long-term records of coral condition and regulatory compliance.

Whereas global climate change is a major threat (Hoegh-Guldberg et al. 2007), efforts must continue to protect coral reefs from local stressors originating in the watershed and coastal zone. This compels use of the most powerful regulatory tools available. The Clean Water Act provides U.S. states and territories the legal framework and regulatory authority to designate waterbody uses specific to coral reef resources, to track status and trends using biological indicators, and to identify impaired waterbodies. Ultimately, the CWA has the capacity to trigger restrictions on diverse human behaviors that could physically damage or send toxics, nutrients, sediment, and pathogens into coral environments. The CWA is a potent regulatory tool with an expressly defined purpose—to protect aquatic life. With defensible scientific underpinnings, the CWA should be at the forefront of U.S. coral reef protection.

Acknowledgments

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Management and monitoring for coral reef conservation in the Port of Singapore

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Abstract. Since its founding in 1819, Singapore has lost an estimated 60% of its coral reef area. Most of this loss is attributed to pressures from land-use necessitated by an increasing population and a fast-growing economy. Nevertheless, high species diversity remains on coral reefs, with more than 250 scleractinian species still extant. This number is more noteworthy as Singapore's total coral area covers only 1000ha. In earlier years, conservation of coral reefs was incidental, contrasting with a more intentional approach today. A major pillar of this new approach involves the setting of strict environmental quality objectives and use of real-time feedback monitoring processes. These rigorous environmental monitoring and management plans allow mitigation of impacts while allowing coastal development that is often necessary in a land-constrained situation, even when these developments are in close proximity to such reefs. In Singapore, the problem of having many stakeholders in coastal areas is exacerbated by the intensity of use resulting from very limited availability of land and coastal areas. Besides traditional coral reef conservation, approaches like active habitat enhancement measures are necessary to ensure long-term sustainability of coral reefs in the face of these pressures.

Key words: Coral, Management, Port, Feedback monitoring

Introduction

Singapore is an island nation with a land area of about 700km^2 and a population of approximately 4.8 million (Statistics Singapore 2008), giving rise to a population density exceeding 6800/km^2. This intense population pressure, coupled with the need to provide a means of income for people represent the socio-economic realities that need to be taken into account when considering conservation needs.

Modern-day Singapore was founded in 1819 by Stamford Raffles of the British East India Company, who recognized its strategic location and natural harbor. Since then, much of the coastline has been transformed from mangroves and coral reefs to one dominated by development, in particular by extensive port facilities in the south of the island. Most of the nation's coral reefs are also found off the southern coasts. An estimated 60% of the original reefs have been lost (Chou 2007).

Impetus to Conserve Marine Biodiversity

Historically, most coral reefs in Singapore were lost due to habitat destruction. Today, factors that could impact coral reefs here include land reclamation, sewage and other high nutrient effluent, industrial effluent (pollutants as well as industrial cooling water), and oil discharges from ships. One major factor is a high level of sedimentation in the water that has a smothering, as well as light attenuation (Chou 2007) impact on hermatypic corals.

In addition, management of coral reefs in Singapore is complicated by the large number of stakeholders: different government agencies hold jurisdiction for different aspects of coral reefs related to the conservation of the latter; private corporations with coastal facilities also have a stake in some coral reefs; nature or recreation groups with coral reef interests also add their voices to the stakeholder community.

In spite of these pressures and complexities in management, coral reefs and other marine habitats have shown resilience and exhibit diversity that can be considered remarkable. More than 250 species of hard corals are present in Singapore; 31 mangrove plant species, together with 11 seagrass species have also been recorded (unpublished data). Fig. 1 shows the locations of coral, mangrove and seagrass habitats in Singapore. While abundances may have declined, species richness can be considered to have remained relatively stable over the last four decades (Chou 2007).

While the continued presence of such rich biodiversity in the light of a worldwide trend of decline is a cause for optimism, it also leads to the responsibility to ensure its conservation is given commensurate consideration.

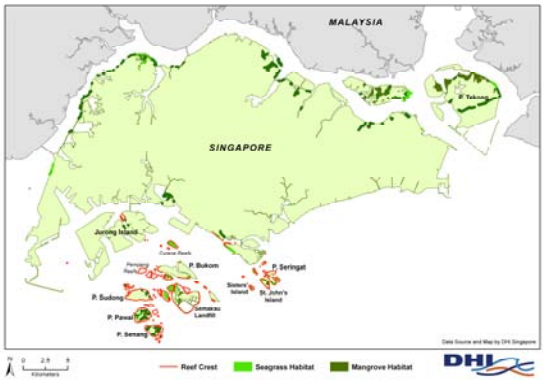

Figure 1: Singapore, showing locations of coral reef, seagrass and mangrove habitats.

Conventional Conservation Approaches Inadequate

Traditional conservation paradigms are largely dependent on protecting areas that possess significant biodiversity by designating such areas as 'no-take' or 'no-go'. In the situation specific to Singapore, i.e., multiple stakeholders coupled with size constraints leading to limited options, intense use-pressures and threats, a more holistic management approach is needed to supplement conventional conservation strategies. This type of approach is likely to be necessary to meet the challenges of conserving coral reefs that occur in close proximity to port or urban centers like Singapore.

Protection of Marine Habitats in Singapore

Prior to 2004, especially in the early days after the founding of Singapore as a port and trading post, there was very limited focus on conservation of coral reefs or other marine habitats. Any government conservation or habitat protection efforts for coral reefs was fortuitous – the result of areas being overlooked in development rather than any conscious motivation to preserve the habitat. One significant milestone related to marine conservation in Singapore occurred in 2004 with the formation of a section dedicated to this purpose in the National Parks Board (hitherto concerned with an almost exclusive terrestrial focus), marking the start of governmental intervention on the part of marine conservation.

In terms of protection of biodiversity from physical development works on coral reefs and other coastal habitats, various international best practices have been adopted in setting out the requirements for approval of such works. For each such development project approved, environmental quality objectives (EQOs), including those related to biodiversity, are set based on current information on the biodiversity at and around the development site that could potentially be impacted ('biodiversity baseline'). The EQOs will typically spell out the level of impact allowed in relation to the project ('no impact' (where no change in the quality or functionality of the habitat if expected using numerical models, and none can be detected in the field) in most cases). Compliance with these EQOs is tracked using monitoring surveys (e.g., by line-intercept transects) and other relevant impact assessment methods (e.g., measurements of water turbidity or sediment settlement). Proposed measures to mitigate any anticipated impacts are also required at pre-approval stage.

In one example of a port expansion project off the southern coast (DHI 2005), which included a land reclamation component, hydrodynamic modeling during impact assessment studies indicated that impacts from the original reclamation profile could be significantly reduced if a different profile was adopted. The original profile was subsequently altered. In addition, the reclamation sequence was optimized to minimize impacts on a nearby coral reef habitat. More than a dozen coral reef sites around the development area are regularly monitored during the period of development, and for 12 months after project completion to ensure that there are no impacts on coral reefs in spite of all the measures imposed. One of the EQOs related to this project was to ensure that suspended sediment coming out of the project site should not exceed a pre-determined level, monitored by turbidity sensors located around the site.

In such projects involving reclamation or dredging where control of suspended sediment is considered important, the conventional approach to control is to impose a cap on dredging or soil dumping rates. Such caps focus on work effort and are presumed to relate to the actual sediment released into the water in a linear and predictable manner. However, this is dependent on a number of assumptions (e.g., on sediment type, current direction and strength, and other ambient environmental factors) that may not hold in all situations. Suspended sediment rates may actually exceed acceptable levels even when contractors are completely compliant with the work effort rate limitations imposed. On the other hand, if the assumptions are overly conservative, the project may drag on longer than actually necessary, leading to impacts caused by the length of time sensitive habitats are subjected to low levels of stress from the development works.

Where it is impossible to prevent destruction of a particular coral reef, every effort is made to minimize the loss of living corals. This is

considered a management imperative, given the very limited extent of coral reefs in Singapore. Controlled translocation of coral reef organisms, with proper monitoring of success (Doorn-Groen et al. 2007) is used in such situations where a coral reef is to be destroyed after consideration of the trade-offs in the decision.

Science-Inspired Approach Towards Long-Term Sustainability of Coral Reefs

Management decisions in Singapore are guided by extensive recent baseline coral reef surveys that cover a majority of the coral reefs here. Regular monitoring is also ongoing for more than a dozen coral reef sites (within a total area of just above 1000ha (unpublished data) of coral reefs in Singapore). This is a very intensive level of study for a very small area; at the same time, hydrodynamic modeling studies covering most of these reefs are also used in management decisions, reflecting the commitment to science-informed decision-making.

Taking advantage of advances in science and technology, a real time suspended sediment feedback system comprising the turbidity sensors mentioned earlier, coupled with a hydrodynamic model incorporating ambient environmental information (Doorn-Groen and Foster 2007) is now used regularly in Singapore. This allows control to be based on the actual level of suspended sediment, rather than on an indirect factor like rate of dredging or soil dumping.

Line-intercept transects are a widely used method of quantifying the abundance (and type) of flora and fauna on coral reefs. In Singapore, results from surveys using line-intercept transects at the reef crest had been considered representative of coral reef health and diversity and used in making management decisions, even though this method only describes a narrow band of life at the coral reef crest. However, studies have shown that on many coral reefs in Singapore, a rich abundance of organisms exists at depths below the reef crest (Goh and Chou 1994, 1995; Goh et al. 1997). As a result of taking such knowledge into account, a new 'Lower Reef Survey' that estimates the rich sponge, ascidian, soft coral and gorgonian fauna has been incorporated in management-related surveys since 2006. This allows survey results used in management decisions to better represent the actual species abundance and diversity situation present on the reefs studied.

Besides stringent measures to enhance the protection of marine habitats (including coral reefs) and the use of existing knowledge of, and advancements in science and technology to enhance conservation management decisions, Singapore has also adopted a management strategy of taking proactive actions to help ensure the long-term sustainability of habitats like coral reefs.

Fragmentation of coral colonies occurs continuously on coral reefs, whether by natural or anthropogenic causes. Based on the foundation of years of research on artificial reefs and recruitment (Chou 2007), a coral nursery was established in Singapore in July 2007 as part of a partnership between the National Parks Board, the National University of Singapore, the National Environment Agency, and Keppel Corporation. Besides studying fragmentation patterns, the project aims to rehabilitate coral fragments collected from reefs throughout Singapore by keeping them at the coral nursery until they are healthy, then transplanting back to natural reefs to enhance natural coral populations.

A study of larval dispersal patterns in Singapore was commissioned to provide information on the genetic connectivity between different reefs in Singapore. Preliminary results from this study are described in Tay et al. (2008). This information will enable management actions (like enhancement transplantation) to be targeted where it will have the greatest impact.

Ultimately, even a large number of individual actions may not have the effect necessary to ensure long-term sustainability of coral reefs in Singapore. An approach to management of coral reefs and other coastal areas that is integrated to include all relevant factors is needed to ensure that such areas can be managed properly. The need for integrated coastal management is well known among scientists and managers globally, and was recently highlighted again by Chou (2007). At the close of this discussion on coral reef conservation within the waters of a busy port, it is good to remind ourselves that while we are mindful of the impacts of port-related activities, like shipping, on sensitive coral reef habitats, an estimated 80% of anthropogenic pollution in coastal areas actually originates from land-based rather than ship-based sources (UNEP GPA 2008).

Conclusions

The limited area, sizeable population, and pace of development in Singapore represent the geographical and socio-economic realities facing conservation proponents.

A holistic approach is needed. In particular, priorities of industry/port development, housing and recreation, and concerns for the protection of coral reefs need to be balanced. This means that some reefs may need to be destroyed in the face of

these societal needs. This also means that 'business as usual' will not be sufficient to ensure the conservation of this globally-threatened ecosystem in Singapore. The intentional, science-based proactive approach discussed above is necessary to ensure the sustained existence of coral reefs in Singapore and other areas sharing similar situations where intense anthropogenic pressures are unavoidable but the will to conserve is present.

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Systematic Marine Conservation Planning: towards a marine representative areas network in Nanggruh Aceh Darussalam, Indonesia

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Abstract (270). A turbulent history and geographic isolation have prevented systematic surveys of coral reef resources in the Indonesian province of Nanggruh Aceh Darussalam (NAD). Following the Indian Ocean Tsunami (IOT), ecological studies revealed that while most marine habitats where unaffected by the tsunami, many reefs were seriously degraded as a result of decades of destructive fishing and poor coastal development. The recovery of these degraded reefs following dramatic reductions in human activity as a result of the IOT is remarkable. Ironically, socio-economic recovery in NAD is re-introducing anthropogenic threats as fishing pressure and coastal development increase. Some conservation initiatives are in progress, however, these also pose a threat because they lack coordination and integration. A systematic marine conservation plan is required to allow natural recovery processes to continue and to protect marine resources from emerging threats. Here, we present a preliminary analysis using 'Systematic Conservation Planning' guidelines in which the conservation goal was to protect at least 30% of existing coastal ecosystems. Data for the analysis included ecological, socio-economic and reef resilience data collected from 64 sites within NAD. Spatial information for the analysis was derived from satellite imagery due to an extended coast line, habitat complexity and variation in accessibility to sites throughout the region. The optimal approach identified was the establishment of many small marine protected areas (MPA) as opposed to a few large areas. This work provides a solid basis for the establishment of a network of MPAs to achieve these conservation goals for the region and protect and enhance livelihoods.

Key words: coral reefs, MPA, marine conservation, tsunami

Introduction

Sumatra has some of the most diverse coral reefs in western Indonesia where the marine fauna, in particular reef fish, includes a unique mix of species from the Indian and Pacific Oceans (Brown 2007). For example, of the 2057 species of reef fish recorded in Indonesia 60% are found in Sumatra (Allen and Adrim 2003)

The province of Nanggruh Aceh Darussalam (NAD) encompasses the northern third of Sumatra. Coral reefs in the province are concentrated in three areas; Pulah Weh and Pulau Aceh off the northern tip of Sumatra; Simeulue Island and Banyak Islands in the south west of the province; plus extensive fringing reefs along the Sumatran mainland (Fig. 1 and Spalding et al. 2001).

Following the Indian Ocean Tsunami (IOT), ecological studies revealed that while most marine habitats where largely unaffected many reefs in NAD had been seriously degraded as a result of decades of destructive fishing and inappropriate coastal development (Baird et al 2005; Campbell et al 2007, Hagan et al 2007). Subsequent research indicates that these degraded reefs have recovered remarkably well, with up to 5 fold increases in coral abundance since 2005, presumably as a result of the dramatic reduction in human activity caused by the IOT (Ardiwijaya et al 2008). Ironically, as socio-economic activity gains pace in NAD anthropogenic threats to reefs, such as fishing pressure and coastal development, are increasing. While some conservation initiatives are in progress, these also pose a threat because they lack coordination and integration.

The aim of this study was to conduct a systematic marine conservation analysis to provide a framework within which the natural recovery processes of the marine environment could continue, and existing marine resources be protected from these emerging threats. Given the preliminary nature of this analysis the objectives and targets were set following discussions among scientists only. Future spatial analysis will include targets and objectives of all stakeholders.

Material and Methods

Conservation spatial planning analysis was conducted using the GIS based tool, MARXAN, integrated with ARCView 3.3 software. Data sources for the analysis included numerous ecological and socio-economic variables, collected between 2006 and 2008 (Table 1).

Ecological data was collected from 64 coral reef sites throughout NAD. As required for MARXAN, we divided variables into 'habitat' layers and 'cost' layers. Habitat layers are those considered when selecting areas for protection and cost layers are those that are likely to affect the costs of including the area in the final spatial solution (Table 1).

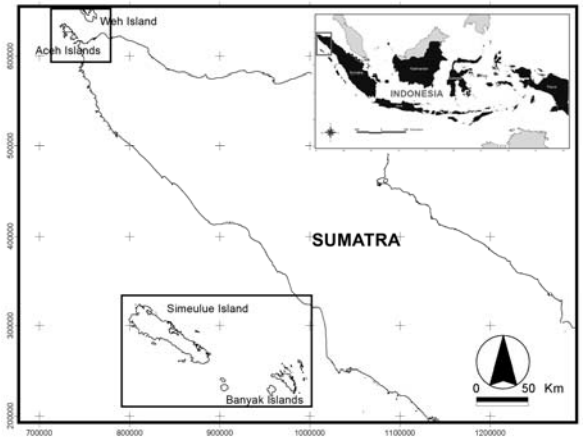

Figure 1. Northern Sumatra with regions of major reef development highlighted by boxes.

Habitat layers	Cost layers
Live coral cover	Distance to village
Coral genera diversity	Distance to port
Coral recruitment	Fishing pressure
Reef fish biomass	Aquaculture
Reef fish abundance	Direct threat (coral mining)
Reef fish species richness	Crown of Thorn Starfish
Clam abundance	
Reef ressilience	
Aggregation sites of Napoleon fish	
Aggregation sites of Bumpedhead parrotfish	
Aggregation sites of Trevaly (*Caranx sp* .)	
Known sites of Manta ray	
Known sites of Whaleshark	
Known sites of Sea Turtle	
Mangrove	

Table 1. A list of the parameters included in MARXAN analysis.

Analysis

For spatial analysis in MARXAN, conservation features were defined by 'bioregion' i.e. a cluster of reefs which have similar characteristics in terms of benthic assemblages and geography (Meerman 2002). This approach was chosen because of the need to analyze many ecological variables (live coral cover, coral diversity, coral recruitment, reef fish biomass and abundance, invertebrate abundance, and reef resilience) based on ranked condition (very good, high, medium, and poor) rather than presence or absence. For the remainder of habitat and cost layers the spatial analysis was performed using presence or absence data.

Spatial data preparation

Quantitative data from the ecological surveys and socio-economic data was converted into four ranked categories (se above). These categories were then incorporated into the previously defined bioregions. Using this approach, each parameter creates four different spatial layers, representing its ranked condition.

Conservation objectives and target

The conservation objectives that we set out to achieve with the analysis were: (i) to conserve 15 to 20% of reef area in total plus (ii) conserve more than 50% of the 'best' habitat. The highest target was set for the best condition for each of the habitat layers representing ecological data and presence or absence of charismatic marine megafauna including Napoleon Wrasse, Bumpedheaded Parrotfish, Trevally, Manta ray, Whale Shark and Sea Turtles. A list of the parameters and targets is presented in Table 2.

Cost Features

Different weightings were given for the four cost values: distance to village, distance to port, fishing pressure (line and net fishing), following the equation:

$$TCF = 2P + 8N + 0.5L + 2V$$

where:

TCF = Total Cost Value
P = Port
N = Net fishing
L = Line fishing
V = Village

The following parameters were 'locked out' to prevent MARXAN selecting areas with these parameters as priority areas; aquaculture, coral mining, and outbreaks of *Acanthaster planci* (COT). Active outbreak levels of COT were defined as an abundance of adult starfish (>15cm in diameter) of over 30 individuals per ha (IUCN, 2004).

Species Penalty Factor

Once the datasets were assembled we set *Species Penalty Factor* (SPF) values for each conservation target. The SPF parameter is crucial for getting useful results from the analysis. The key point is that SPF values must be chosen so that penalties for missing conservation targets are scaled appropriately relative to each other term in the objective function (Ardron et al 2008). SPF helps MARXAN to define the priority level for each conservation target/feature when selecting priority areas.

We set SPF values to range from 0-100 for all parameters and set a value of 100 for several of the highest biodiversity conditions (Table 2).

Boundary Length Modifier

The Boundary Length Modifier (BLM) is used to improve the clustering and compactness of individual solutions (McDonnell et al. 2005). Higher BLM values mean that selected areas will be more clustered in order to reduce the cost of boundary. In this analysis we set BLM value to 10 to create clustered selected areas.

No.	Parameters	Target	SPF
1	Mangrove	17.00	80.00
2	Live coral cover Low	0.00	10.00
3	Live coral cover Med	72.00	50.00
4	Live coral cover High	100.00	100.00
5	Coral recruit Low	50.00	10.00
6	Coral recruit Med	20.00	50.00
7	Coral recruit High	30.00	50.00
8	Reef fish biomass Very low	0.00	10.00
9	Reef fish biomass Low	0.00	30.00
10	Reef fish biomass Med	80.00	50.00
11	Reef fish biomass High	100.00	100.00
12	Coral diversity Very low	0.00	10.00
13	Coral diversity Low	0.00	10.00
14	Coral diversity Med	53.40	50.00
15	Coral diversity High	70.00	100.00
16	Clam abundance Low	0.00	10.00
17	Clam abundance Med	81.00	50.00
18	Clam abundance High	90.00	80.00
19	Bumpedhead parrotfish	90.00	50.00
20	Trevaly	200.00	50.00
21	Napoleon fish	30.00	75.00
22	Manta ray	50.00	50.00
23	Sea turtle	50.00	75.00
24	Whaleshark	28.80	50.00
25	Village	174.30	50.00
26	Reef fish abundance Very low	0.00	10.00
27	Reef fish abundance Low	10.00	10.00
28	Reef fish abundance Med	73.80	50.00
29	Reef fish abundance High	50.00	100.00
30	Reef resillience Low	0.00	10.00
31	Reef resillience Med	101.10	50.00
32	Reef resillience High	19.20	100.00

Table 2. Target and Species Penalty Factor

Results and Discussion

Reef condition on Pulau Weh was the best in the northern region of NAD giving MARXAN many options for the placement of protected areas indicated in pink in Fig. 2. Dark purple areas were excluded from the analysis ('locked out') because of their proximity to ports and/or dense urban populations. Areas in brown were also excluded because these areas are already under management.

In Pulau Aceh (Fig. 3), MARXAN select only 10% of reef area for protection. Sites in Pulau Aceh generally have much poorer coral reef condition when compared to other areas within the region (Baird et al. 2005; Campbell et al. 2007). In this analysis, MARXAN selected areas with the highest coral generic richness, reef fish abundance and resilience. The purple color indicates areas excluded due to proximity to the port.

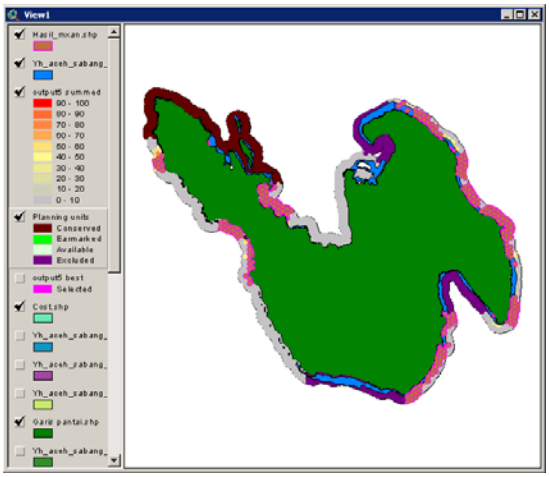

Figure 2. Analysis result of Pulau Weh– northern NAD.

In Pulau Simeulue (Fig. 4), less than 3% of the coastal area was selected by MARXAN. In general, Pulau Simeulue had relatively good reef in some areas, but this result may be affected by limited data. Pulau Simeulue has approximately 300km of coastline and consequently a comprehensive dataset on reef condition is not available.

In Pulau Banyak (Fig. 5), approximately 40% of the coastal area was selected for protection by MARXAN. Areas excluded from the analysis due to proximity to the port or sites of coral harvesting are indicated in red (Fig. 6).

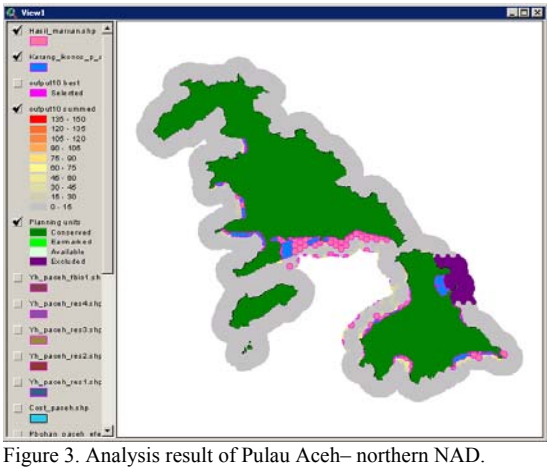

Figure 3. Analysis result of Pulau Aceh– northern NAD.

Conclusion

The analysis identified a ready solution to achieve the limited goals of this preliminary study, i.e. 15 to 20% of reef area conserved in total including more than 50% of the 'best' available habitat. The exclusion of

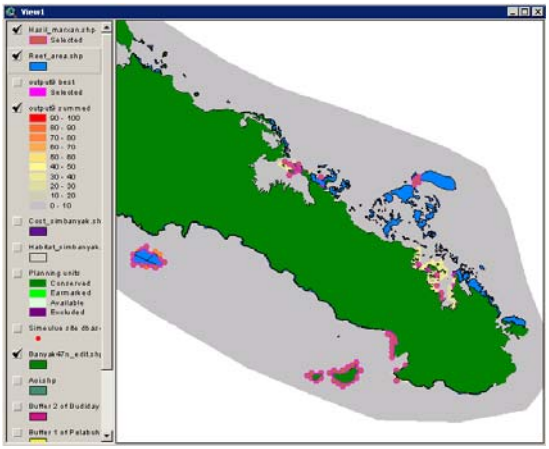

Figure 4. Analysis result of Pulau Simeulue– south-western NAD.

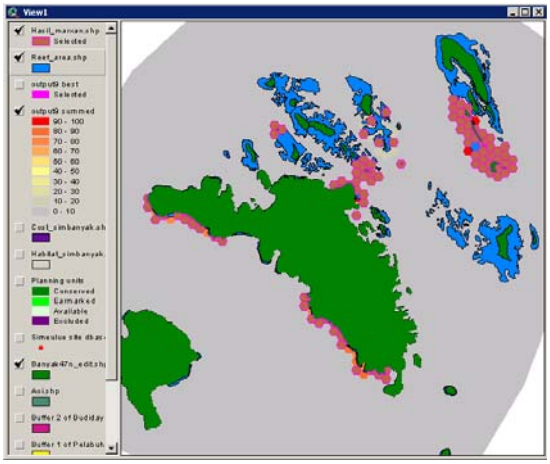

Figure 5. Analysis result of Pulau Banyak– south-western NAD.

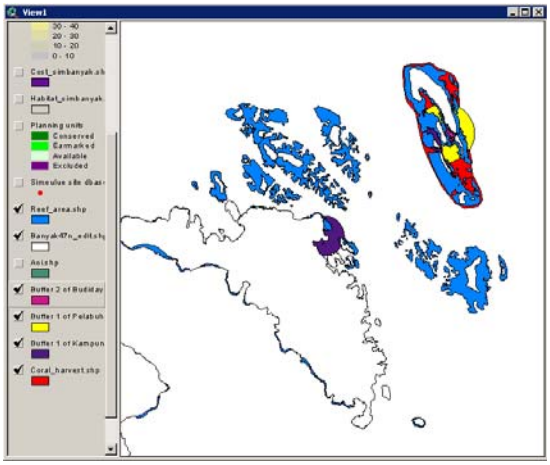

Figure 6. Areas excluded from the MARXAN analysis due to pressure from coral harvesting (indicated by red color).

areas close to ports, high population pressure and conflicting resource use suggests this spatial plan would result in little disruption to other marine resources users and therefore may be a practical solution to the province of NAD's conservation obligations. We must, however, stress that this is a 'first look' conservation plan. Future analyses must seek to consult with other stakeholders to set appropriate targets to achieve a balance between conservation and development in the marine environment in NAD.

Acknowledgements
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Fishery management for artisanal reef fisheries in developing countries: A holistic economic approach

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Abstract. Traditionally exploited renewable resources in developing countries, in particular artisanal coral reef fisheries, are often difficult or impossible to manage with classical fishery management tools. This paper argues that using the economic perspective to take a closer look at the special characteristics of these fisheries and the decisions that underlie the behavior of the fishing households, a different approach to management reveals itself. A set of feasible policy options, based on a broader economic scale, can be used to indirectly manage for sustainable fishing levels. We summarize some basic economic concepts for a non-economic audience, and apply them to the artisanal reef setting. The central policy implication of these models is that aligning actual incentives faced by fishers with the conservation objective is critical for success (though by no means sufficient). The central incentive "not to fish" is raising the opportunity cost of time of fishing households. Empowering fishers to pursue higher incomes beyond fishing will reduce the effective pressure on the resource. Hence any policy facilitating alternative livelihoods, increasing education or skills, or providing investment grants or credit should be considered conservation policy in and of itself. Further, long-term family planning policies are likely to have the greatest impact.

Key words: Economics, small-scale fisheries, resource management, alternative livelihood, labor supply

Introduction

Most coral reefs are located in developing countries and support a large number of rural fishing households. These labor-intense, small-scale, traditional, multi-species, coastal fisheries---in short artisanal fisheries---are the primary use of the reef. With population growth, development, and globalization these resources are facing tremendous pressures, and their fate rests, to a large degree, on the economic behavior of the fishing households. Unless policy makers intervene directly or indirectly in the decisions of these households, many of these resources will be depleted and possibly permanently damaged or destroyed.

Since the artisanal fishing households are often dependent on the resource and due to their large numbers, failure to sustainably manage these fisheries will have significant impacts beyond purely conservation and fishery concerns. A collapse will create unemployment, poverty, and possibly lead to large population movements. Because the human dimension is so dominant in these artisanal fisheries, it has been argued that they require a modified policy perspective (Tietze et al. 2000; Garcia et al. 2008).

A common characteristic of these resources is that they are being exploited on an open access basis. As bioeconomic theory and plenty of experience have shown, an open access resource will almost inevitably be depleted over time, unless policy makers intervene (McGoodwin 1990). Yet effective resource management is often neither feasible nor practical in a poor developing country setting.

For a variety of reasons, artisanal reef fisheries in poor developing countries are often difficult or impossible to manage with classical resource management tools. This paper is primarily concerned with these situations where successful management, i.e. access limitation of any sort, is not possible. We argue that by taking a more holistic economic approach to the behavior of the fishing households, a different set of policy options becomes apparent. These feasible policies would indirectly manage for a sustainable level of fishing effort. They could potentially conserve the biological health and potential of the resource in spite of it being exploited under an open access property rights regime. The paper is intended to make the case to a broad audience for more involvement of the science of economics when designing conservation policy for reef resources in developing countries. The economic perspective can offer an important understanding of the human actors in these settings.

Bioeconomics and Artisanal Reef Fisheries

Historically, the management of a fishery was treated as a biological exercise. Fishery economics, the bioeconomic approach to renewable resource extraction, began in the 1950s. Seasonal closures,

specific gear restrictions, and other regulations which did not account for human behavioral adaption often further exacerbated the "race for fish" (Homas and Wilen 1997). Economists point to the lack of private or common property rights (conferring the exclusive right to use a resource) as the fundamental culprit of this waste and to market-based tools, such as individual transferable quotas, as the solution. However, the practical importance of this work is limited for the management of artisanal reef fisheries in poor developing countries. First, in many such countries fishery management, in its narrow sense, is simply not feasible. And second, the settings and assumptions that drive the bioeconomic framework often do not apply.

The introduction of effective, modern management to artisanal reef fisheries in many poor developing countries faces many challenges. The first hurdle is a lack of recognition of the actual scarcity of the resource and no legal entity with clear management responsibility. Even if the need for management is recognized, few of the needed financial and administrative resources exist, and there are a plethora of more pressing problems (e.g. acute poverty, disease). The remoteness of locations, the lack of a property rights tradition, and minimal law enforcement make introducing most fishery management tools very difficult. Limited or non-existent data on catch, effort, and the resource status is a further problem. Finally, the costs of creating and running such a system would likely be prohibitive relative to the value of the fishery and the means of many poor developing countries.

Situations where successful management of artisanal reef fisheries is more likely to be feasible---not the primary concern of this paper---are richer developing countries where local marine tenure traditions exist and have government support. If access can be limited, protected areas are of particular promise because they can take account of many of the special characteristics of artisanal reef fisheries.

The bioeconomic framework and the associated management tools are primarily developed for an "industrial" fleet fishing a large single species stock, with a focus on capital investment in vessels and equipment. Fishers are modeled as profit maximizing firms active in perfect markets for capital, labor, and fish. Total fishing "effort" is usually dominated by vessel and gear capacity, with labor playing a secondary roll. All of these assumptions are in stark contrast to the settings found in artisanal reef fisheries in developing countries.

Artisanal fishers are usually small operations in a local, seasonal, multi-species, multi-gear fishery, where single species management is impossible. They use predominantly small wooden boats and basic gear (lines, nets, spears, etc.). Human labor is the dominant input, with capital playing a secondary role. The artisanal fishing households often live in poverty and consume the less-marketable portion of their catch. Further, market failures plague these fisheries. Product markets exhibit high transaction costs due to the remoteness of small fishing communities and the perishable nature of fish, while labor markets often do not exist (Polunin and Roberts 1996).

It should be noted that semi-subsistence, agricultural households, including fishing households, are often characterized as stagnant entities. This is usually far from the truth as they engage in occupational multiplicity and respond strongly to changes in prices and costs. They behave as economic agents, suggesting that fishing is best understood as one productive activity among many (Tietze et al. 2000; Allison and Ellis 2001; Liese et al. 2007).

More recently outboard motors have extended the range of the typical fisher, while monofilament nets have increased the intensity of exploitation. As a result, the effective fishing capacity has increased, but the overall fishing effort in most artisanal reef fisheries is still primarily a function of the labor supplied by the households. Given the predominance of the human dimension, preferences, market aspects, and socio-economic and demographic variables must be taken into account when designing policy. While the bioeconomic approach offers little practical guidance, the economic approach in general can be very helpful.

Effort in an Artisanal Reef Fishery

Given a level of technology, the total effort in a traditional setting can be decomposed into individual and population elements (Kalland 1995). Both are determined by household behavior, yet the relevant decisions are particular to each level. Fig. 1 is a

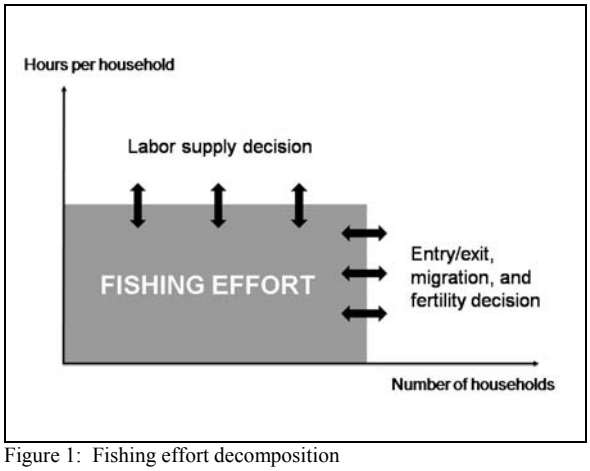

Figure 1: Fishing effort decomposition

stylized illustration of this decomposition of total fishing effort. The representative household's fishing

effort times the number of fishing households leads to the total fishing effort, which is depicted as the shaded area. The individual element is the fishing effort decision by each household, i.e. the intensity of use or hours spent fishing. The population element comprises occupational entry/exit, migration, and fertility decisions. Another way to look at Fig. 1 is as a split along temporal lines. Since traditional fishers usually are independent entrepreneurs, in the short run, the total effort changes due to changes in fishing intensity by existing fishers. Over a longer time horizon, entry/exit into the population of fishers becomes the dominant determinant of the total effort.

Economic Modeling of Labor Supply

The effort decision by a fisher can be modeled in the utility-theoretic framework of micro-economic theory. At the most basic, an individual's utility depends on the consumption of two normal goods, one leisure and one a catch-all for purchased consumption (see any microeconomic text for a more thorough discussion). The individual maximizes his utility by selling part of his fixed endowment of time (i.e. giving up leisure) at a fixed wage rate in order to generate an income with which to buy the consumption good. The amount of labor sold---the labor supply---depends on the wage rate. The wage rate influences the supply through two channels: 1) as the price at which consumption can be substituted for leisure, leading to a substitution effect, and 2) as a determinant of the total wealth (time endowment valued at the wage rate), leading to an income effect. The substitution and income effects have countervailing effects on the labor supply. If this analysis is expanded to include a minimum subsistence requirement it gives rise to the inverted "S"-shaped labor supply function depicted in Fig. 2.

The traditional portion of the labor supply curve is upward sloping, where an individual substitutes consumption for leisure as the wage increases, i.e. as the opportunity cost of consuming leisure increases. In this area, the substitution effect dominates. At higher wage rates the income effect starts to dominate the substitution effect. Starting at this point the labor supply curve becomes backward bending, which is characteristic of richer individuals or economies. If an individual is secure in his subsistence requirement, i.e. income beyond labor income, then his labor supply curve begins with the upward sloping segment. The wage that corresponds to the lowest point is referred to the reservation wage and at wages below this level, the individual will choose not to participate in the labor market.

The "distress sale" portion of the curve would only be observed in a traditional sector of a developing country in the absence of any type of social insurance programs (Sharif 2000; Dessing 2002). This portion of the labor supply curve results if individuals face serious poverty where their only income is through labor, and the wage is less than a living wage. Along this section of the curve an individual only sells his labor due to acute distress. As the wage rises, the individual reduces his supply of labor, as less is necessary to generate the minimum subsistence income. In the fishery context this might reflect a Malthusian overfishing scenario (McManus 1996).

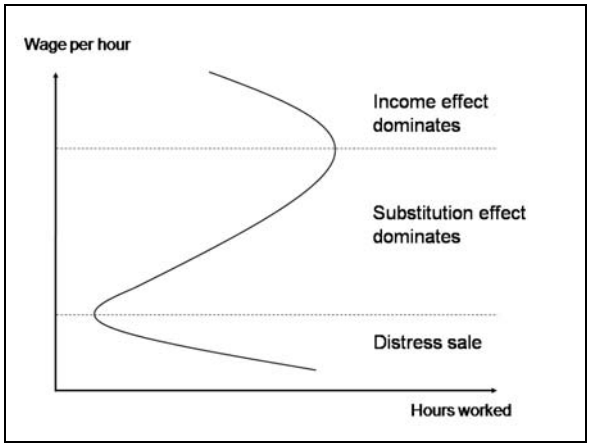

Figure 2: Complete neo-classical labor supply function

In a rural setting of a developing country, few individuals work for a fixed wage as most are self-employed. It seems reasonable that the above neo-classical labor supply relation still explains behavior if one reinterprets the wage as the effective return to labor of a productive activity (net of non-labor costs). For example, the amount of time a person would spend fishing would depend on the implicit wage of the activity, i.e. the productivity of fishing times the price of fish. Differing implicit wages would give rise to a schedule of labor supply roughly corresponding to Fig. 2. The key idea is that developing country households are surrounded by opportunities and challenges in their daily struggle to make a living. In the informal village economies of most artisanal reef fishers, psychic, physical, and material benefits and costs play the same role as financial benefits and costs do in a more formal economy. These incentives drive behavior.

An important disincentive for spending time on any one activity is the opportunity cost of time, which is the value of the time in its next best use. Regardless of whether labor markets exist or not, each individual (or household) faces an opportunity cost of time, be it an implicit wage, an actual wage, or a value for leisure. The possibility of engaging in multiple activities, as is very common in developing countries, can be economically modeled with the help of the time allocation or home production frameworks (Gronau 1977).

Finally, the population of fishers is partly determined by occupational entry/exit decisions. Households enter the fishing enterprise when economic incentives are right and exit when more income (or utility) can be generated in a different profession (Cinner et al. 2008). Entry/exit decisions can be modeled with approaches similar to the ones mentioned above for labor supply and time allocation.

Migration and Fertility

In the long run, migration and population growth are driving forces behind the exploitation of renewable resources (Kramer et al. 2002). These are complex phenomena, and it is a multi-disciplinary undertaking to define, measure, and explain reasons for and consequences of demographic change. In spite of this, population growth must at least be considered, especially since resource conservation is about this long run. Migration among fishers is especially important since it re-allocates fishing effort over space. An important and successful approach to understand and explain population movements is the economic one by framing migration as the outcome of a utility maximizing decision by individuals. At the end of the 19th century E.G. Ravenstein developed six "laws" of migration which have stood the test of time. One of them is "the dominance of the economic motive" for migration (Todaro 1976).

Fertility and mortality are additional forces changing the fishers population level. In the past, fertility has often been the dominant force of change. Population growth without economic development leads to pressure on the natural resources. Even if access is limited, population pressure in such settings has a high potential to undermine any management institutions. Artisanal fisheries are particularly prone to this, as it is ethically questionable to exclude people living at the existence minimum from a potential food source. Examining the fertility decision by households and linking it to economic development is a major topic in demographics and population economics (Becker 1960).

Implications for Conservation Management

Fisheries management is foremost about managing people rather than fish (McManus 1996). As a result, the economic approach---the utility theoretic framework---is central to evaluating the design of any policy aimed at conservation in an artisanal reef fishery. The most important economic contribution to management of reef resources is focusing the discussion on incentives---the factors encouraging, motivating, and inducing behavior.

For any policy to be successful it must align the incentives the fishers face with the conservation objective. Any policy that changes the incentives faced by the fishers by changing relative prices and costs within the fishery will impact the resource status. Since these incentives can be influenced by a variety of non-fishery policies, indirectly managing for a sustainable fishing effort level can be possible. Non-fishery policy instruments in areas such as development, employment, population, and social policy could be tried. Unlike with standard bioeconomic prescriptions, removing the open access nature of the fishery is not necessary. McGoodwin (1990) refers to such policies as "passive indigenous regulation," though he also includes anthropological concepts, such as customs and beliefs.

The central incentive "not to fish" is a high opportunity cost of time among the resource users. In particular, helping fishers find better paying livelihoods raises their opportunity cost of time and will reduce the pressure on the resource. The scope and scale of the labor market can be expanded by creating alternative employment or income diversification opportunities. Other interventions include increasing the education level, providing skills, or raising productivity in non-fishing industries or agricultural (intensification).

The above is related to the alternative livelihood approach, but with the emphasis on fishers being "pulled" out of their profession by better opportunities elsewhere rather than being pushed out of fishing and needing to find other employment. Creating alternative income opportunities should be seen as a conservation policy in itself, rather than just as an add-on to mitigate unwanted side effects of a conservation policy. Policies that empower households and enable them to pursue alternative livelihoods, rather than prescribing specific ones, are likely to be more effective. In addition to education and skills mentioned above, market liberalization, land reform, and providing credit or grants for investment beyond fishing are policies of this nature.

Other policies likely to increase the opportunity cost of time include general development and poverty reduction. With increasing wealth, households will value leisure time more. New entertainment options might also increase the value of leisure time directly. Reducing poverty through income redistribution would probably have similar effects. But caution is warranted. Policies that promote general development will likely also create incentives for households to fish more. For example, improved infrastructure or exposure of artisanal fishers to regional and global product markets can increase fish demand, raise its price, and hence lead to more exploitation (Cinner 2006; Liese et al. 2007). Anticipating the various incentives and disincentives created by a policy and the resulting impact on the resource might be called a holistic economic approach to resource management.

Demographic phenomena are always very context dependent, so general insights for conservation policy are limited. We might risk speculating that policies that set the incentives for households to discourage fishing are likely to have similar effects on migrants' fishing decisions and by extension on the migration decision (if it is motivated by fishing income, as it often is). On the other hand, family- or refugee-based migration is clearly beyond simple economic analysis. Over time, fertility is the central determinant of fishing effort in a poor developing country setting. Three general policy implications suggest themselves: 1. Family planning policies are among the most powerful conservation measures available; 2. any policy with the aim to conserve the reef resource over time must anticipate and be able to withstand demographic developments; and 3. if population growth and in-migration cannot be stopped, access must be limited or the resource will be depleted.

Conclusion

This paper sets out to make the case for more involvement of the science of economics when designing conservation policy aimed at coral reef fisheries in developing countries. This brief paper is in no way a comprehensive or exhaustive review or discussion of this topic. Instead it summarizes some basic economic concepts for a non-economic audience, and applies them to the coral reef setting.

We describe the science of fisheries economics and its serious limitations when applied to a developing country artisanal reef fishery. Instead, we propose that more basic and usually simpler economic models can be more helpful---and in fact critical---for understanding effort in these fisheries. Labor supply and time allocation models can be employed to help explain households' fishing behavior. The determinants of the household's fishing decision can sometimes be influenced by policies, thereby providing possible avenues to indirectly manage for conservation of the resource, even if classical fisheries tool are impossible to implement (which is often the case).

The central implication of these conceptual economic models is that aligning the actual incentives faced by fishers with the conservation objective is a necessary condition for success (though by no means sufficient). In many cases the only feasible incentive "not to fish" is raising the opportunity cost of time of the fishing households. Empowering fishers to pursue higher incomes outside of fishing will reduce the fishing pressure on the resource. Hence promotion of alternative livelihoods, education, skills, and others mentioned should be considered conservation policy in and of itself.

Finally this paper argues that conservation policies that aim for sustainability must take into account the very long run. Over time, demographic developments will usually be the dominant force determining fishing effort in a developing country setting. Conservation policies that ignore demographic pressures are doomed to failure by (lack of) design unless access can be effectively limited.

Acknowledgement

The views expressed herein are my own and do not necessarily reflect any position of the Government or the National Oceanic and Atmospheric Administration.

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Contaminants in Fishes from Johnston Atoll

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Abstract. This study examined the distribution of military-industrial contaminants accumulating in coral reef fishes in the lagoon of Johnston Atoll, Pacific Ocean. This atoll was a major military base involved in nuclear and chemical weapons as well as being a depot, transient airfield and harbor since the 1930's. The base was closed and abandoned in 2003. Fishes of different trophic levels were sampled from locations throughout the atoll. Contaminants of concern included radionuclides, heavy metals (antimony, arsenic, barium, cadmium, chromium, copper, lead, mercury and zinc) and organic contaminants including; polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), herbicides, dioxins and furans (PCDD/PCDFs). The northwest section of Johnston Island, the largest island in the atoll, was the area with the highest level and variety of contaminants in fishes and sediments. This was near the site of the open burn pit and trash dump, fire training and explosives detonation areas, and the former storage site of Herbicide (Agent) Orange.

Keywords: Central Pacific Ocean, PCBs, PAHs, dioxin, Agent Orange, weapons grade plutonium

Introduction

Johnston is a remote Central Pacific Atoll. The nearest landfall is French Frigate Shoals, 804 km (500 miles) north. The atoll is about 1,287 km (800 miles) southwest of Honolulu, Hawaii and 1,440 km (900 miles) north of the Line Islands of Kiribati. Johnston Atoll (JA) came under military control in 1934 and remained so until 2003. Its use varied during those years but included extensive use as a refueling site, atmospheric nuclear testing, master LORAN station for the Pacific, storage site for unused herbicide orange (agent orange) and chemical weapons, and the incineration of chemical weapons in the Johnston Atoll Chemical Ammunition Disposal System (JACADS) (Lobel and Lobel 2008).

Many of the activities mentioned above, as well as infrastructure needed to support a military and civilian workforce of up to 2000 people, contributed to soil and sediment contamination within the atoll (Lobel and Kerr 2002). Polycyclic aromatic hydrocarbon, petroleum hydrocarbon and metal contamination was associated with refuse burning, fire training operations and fuel storage. Organochlorine contamination due to leakage from discarded electrical equipment, transformers, polychlorinated biphenyl (PCB) contaminated fuel and herbicide orange (HO) were the main contaminants of potential concern (COPC) due to accumulation in top predators and potential toxicity. HO contains two active ingredients, the n-butyl ester of 2,4- dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), as well as the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

Polychlorinated biphenyls were found mainly in two areas of the lagoon, the west end of Sand Island and in the Navy Pier (tank 49 lagoon) area on Johnston Island (JI), the main island within the atoll. Polychlorinated dibenzo-p-dioxin and dibenzofuran (PCDD/PCDF) contamination in soil and nearshore sediments of the northwest corner of JI was caused by leaking storage drums containing HO (Lobel and Kerr 2002).

Of the four aborted nuclear tests that occurred on JI, two would have contributed to the dispersal of radionuclides into the lagoon. Most of the debris and residual plutonium from the STARFISH event, aborted at 30,000 feet, landed in the water surrounding JI and on adjacent Sand Island. The BLUEGILL PRIME event scattered radioactive material primarily downwind of the launch emplacement. The single residual contaminant was Weapons Grade Plutonium (WGP), which consists of five isotopes of plutonium (238, 239, 240, 241, and 242) and americium-241.

This review summarizes contaminant data in aquatic biota from multiple studies/sampling events sponsored by different agencies. Management responsibility for JA is shared among several agencies including the US Air Force, US Army, US Coast Guard, US Fish and Wildlife Service and the Defense Threat Reduction Agency thus, an integrated approach to ecosystem management including the need for long-term monitoring and potential cleanup is necessary.

Materials and Methods

Samples were collected from sites with known sediment contamination as well as sites with no sediment contamination for comparison (Lobel and Kerr 2002). The main concerns were PAHs, PCDD/PCDFs and metals in fishes from the NW corner of JI and down wind from JACADS, PCBs from the Tank 49 lagoon and Sand Island, radionuclides surrounding JI and Sand Island. Fish sampled from Kaneohe Bay, Oahu, Hawaii served as a reference site for radionuclides.

Sample species were chosen based on food habits or potential for accumulating contaminants, relative abundance and distribution within the atoll. Species sampled included parrotfishes *Chlorurus sordidus, Scarus psittacus, S. rubroviolaceous*; surgeonfishes *Ctenochaetus strigosus* and *Acanthurus triostegus*; the damselfish *Abudefduf sordidus*; goatfishes *Mulloidichthys flavolineatus Parupeneus multifasciatus, P. trifasciatus;* the triggerfish *Rhinecanthus aculeatus;* and the squirrelfish *Sargocentron tiere.*

Fish samples were collected by spear. Individual fish were placed in plastic bags after capture. Each sample location was mapped using GPS then plotted on nautical charts and high definition aerial photographs. Samples were chilled on ice immediately upon collection. Fish were identified to species, weighed and measured (SL). Samples were again cooled to 4°C prior to shipment on dry ice. Quality control samples consisted of approximately 10% of the total samples sent for analysis. Splits, duplicate samples, matrix spikes as well as field, trip, and laboratory blanks were used to determine the precision and accuracy of the analytical data. All analyses were completed by government contract laboratories using US Environmental Protection Agency (EPA) approved methods (USEPA 1993, 1994,1996, 1999; SW-846 method number in parentheses; http://www.epa.gov/sw-846/main.htm). Samples were extracted and separated using methods 3500, 3540(C) and 8000. Chlorinated herbicides were measured with gas chromatography (GC) using methylation (8150). Individual congeners of polychlorinated biphenyls (PCBs) were measured using high resolution GC and low resolution mass spectrophotometry (HRGC/LRMS; 8082A, modification 680 and EPA Method 1668). Total PCBs were taken as the sum of all mono- to decachlorinated congeners. Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were measured using HRGC/HRMS (8290). Toxicity equivalents (TEQ) for 2,3,7,8 tetrachlorodibenzo-p-dioxin were calculated using fish derived toxicity equivalency

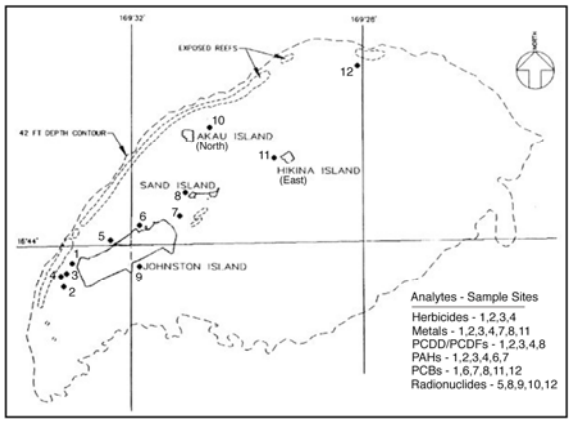

Figure 1. Biota sampling sites within Johnston Atoll. 1) offshore of the former Herbicide Orange (HO) storage area, 2) west camera stand, 3) across the channel from the HO site, 4) inner reef area, 5) offshore of the radiation control area (RCA), 6) tank 49 lagoon area, 7) Buoy 14, 8) west end of Sand Island, 9) south side of Johnston Island, 10) Blue Hole, 11) East Island, 12) Donovan's Reef.

factors (TEF) for 17 2,3,7,8 substituted congeners (Van den Berg et al. 1998). Polycyclic aromatic hydrocarbons (PAHs) were measured with GC/MS (8270). Barium, cadmium, chromium, copper, lead, and zinc were measured using atomic absorption (AA) with direct aspiration (7080, 7130, 7190, 7210, 7420, 7950) while antimony and arsenic used a borohydride reduction (7062). Mercury was measured using the cold vapor technique (7471A).

Radionuclide analysis was conducted by Oak Ridge National Laboratory (ORNL), Grand Junction, Colorado. Whole fish bodies without viscera and the viscera were analyzed separately by alpha spectrometry for ^{241}americium, ^{244}curium, ^{238}plutonium, $^{239+240}$plutonium, and ^{242}plutonium using ORNL procedures PU242 and RC-19 R06.

Results

Multiple sampling events occurred between 1995 and 2003 focusing on different areas of the atoll and differing contaminants (Fig.1).

In total, 94 reef fish samples were analyzed for PCBs, 80 for PCDD/PCDFs and metals, 55 for the herbicides 2,4-D and 2,4,5-T, 65 for PAHs, and 82 for radionuclides.

The herbicides 2,4 D and 2,4,5 T were not detected in any fish tissues. Trace levels of PAHs were detected in fish tissues from sites 1,2,3,4 and

6 but all detections were below the limits of quantification.

The most toxic dioxin congener, 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) was detected in 35% of the 80 fish samples analyzed for PCDDs/PCDFs. Of these samples 79% (27 of 31) of the detections were from offshore of the former

Table 1. Distribution and concentration of A) organic constituents including percent lipids, B) metals and C) radionuclides in Johnston Atoll reef fish tissue and viscera by site. Sample sizes (N) are the number of individual fish analyzed with the number of species collected at each site shown in parentheses. Data are summarized as mean ±sd (range) wet weight (ww). Sample sites are shown in Fig. 1.

A – Organic concentrations (whole body, pg/g or ng/g ww)

Site	N	TCDD (pg/g)	TEQ (pg/g)	∑PCBs (ng/g)	Lipid (%)
1	31 (8)	6.3 ± 8.8 (0.6-40.5)	5.5 ± 8.4 (0-40.6)	35.0 ± 27.6 (6.8-77.5)	3.6 ± 1.4 (1.6-5.2)
2	13 (5)	ND	0.02 ± 0.03 (0-0.08)	4.1 (N=1)	3.8 ± 3.4 (0.7-11.2)
3	12 (6)	0.82	0.2 ± 0.7 (0-2.4)	NA	3.6 ± 3.1 (0.8-8.7)
4	6 (4)	0.4	0.1 ± 0.2 (0-0.5)	NA	4.5 ± 2.4 (1.4-7.8)
6	35 (5)	NA	NA	1432 ± 1538 (52.4-6789)	3.5 ± 1.8 (0.6-9.0)
7	18 (1)	ND	ND	36.6 ± 52.3 (3.9-230.0)	3.5 ± 2.0 (1.1-9.3)
8	18 (1)	ND	0.04 ± 0.03 (0-0.08)	753.5 ± 900 (29.7-3270)	3.9 ± 1.4 (1.8-7.1)
11	12 (1)	ND	0.0009	12.8 ± 9.2 (3.4-38.7)	4.0 ± 2.1 (1.7-8.0)
12	8 (1)	NA	NA	15.1 ± 31.0 (3.7-91.8)	2.7 ± 2.2 (0.6-6.0)

B – Metal concentrations (whole body, µg/g ww)

Site	N	As	Ba	Cd	Cr	Cu	Hg	Sb	Pb	Zn
1	31 (8)	2.0 ± 0.2 (1.8-2.3)	1.0 ± 0.3 (0.6-1.3)	0.04 ± 0.02 (0.03-0.08)	0.6 ± 0.5 (0.3-1.5)	0.6 ± 0.1 (0.5-0.7)	0.03 ± 0.01 (0.02-0.05)	0.01	0.5 ± 0.2 (0.2-0.9)	15.7 ± 4.3 (11.0-22.5)
2	13 (5)	2.1	13.2 ± 7.4 (1.0-19.4)	0.3	0.4	0.7	0.02	ND	0.07	15.0 ± 8.5 (5.8-34.2)
3	12 (6)	ND	13.7 ± 2.0 (12.4-16.0)	ND	ND	ND	0.05	NA	ND	15.1±10.10.1 (6.7-43.1)
4	6 (1)	ND	15.8	ND	ND	ND	ND	NA	ND	14.1± 6.9 (7.8-24.2)
7	6 (1)	2.0 ± 0.8 (1.3-3.4)	1.4 ± 0.6 (0.7-2.3)	0.08 ± 0.05 (0.03-0.2)	0.3 ± 0.1 (0.2-0.4)	0.6 ± 0.1 (0.5-0.7)	0.02 ± 0.001 (0.02-0.03)	ND	0.5 ± 0.3 (0.2-1.0)	19.1 ± 3.4 (12.5-22.1)
8	6 (1)	2.2 ± 0.5 (1.6-2.8)	0.8 ± 0.5 (0.4-1.8)	0.04 ± 0.01 (0.02-0.1)	0.4 ± 0.1 (0.4-0.6)	0.7 ± 0.2 (0.6-1.0)	0.08 ± 0.04 (0.03-0.1)	ND	1.7 ± 2.1 (0.6-6.0)	20.7 ± 5.1 (16.1-28.4)
11	6 (1)	2.6 ± 0.2 (2.3-2.9)	0.5 ± 0.2 (0.2-0.7)	0.1 ± 0.05 (0.05-0.2)	0.5 ± 0.3 (0.4-1.1)	0.7 ± 0.05 (0.7-0.8)	0.1 ± 0.1 (0.04-0.3)	ND	0.1 ± 0.05 (0.1-0.2)	18.5 ± 2.6 (15.0-21.6)

C – Radionuclide concentrations (pCi/kg ww)

		^{238}Pu (pCi/kg)		$^{239+240}$Pu (pCi/kg)		^{242}Pu (pCi/kg)		^{241}Am (pCi/kg)	
Site	N	Body	Viscera	Body	Viscera	Body	Viscera	Body	Viscera
5	25 (5)	0.2 ± 0.1 (0-0.4)	7.7 ± 13 (0-55)	2.9 ± 3 (0.4-10)	481 ± 805 (4.9-3816)	ND	3.0 ± 1 (1.5-4.3)	0.4 ± 05 (0-1.4)	77.9 ± 147 (0-654)
8	15 (3)	ND	3.7 ± 2 (0-6.6)	0.4 ± 0.04 (0-0.4)	26.4 ± 18 (0-60.1)	0.2	1.8	ND	11.4 ± 5 (4.5-20.2)
9	10 (1)	0.2	2.0	0.7 ± 0.3 (0-0.9)	13.6 ± 6 (0-28.7)	ND	1.4	ND	6.6 ± 2 (0-8.7)
10	10 (1)	0.3 ± 0.01 (0-0.3)	ND	ND	5.3 ± 2 (0-7.8)	ND	2.1 ± 0.5 (0-2.5)	0.3 ± 0.03 (0-0.3)	3.6 ± 1 (0-5.2)
12	25 (6)	0.5	4.2 ± 3 (0-8.6)	0.7 ± 0.5 (0-1.5)	6.3 ± 4 (0-10.1)	0.3	ND	0.5	5.3 ± 2 (0-7.4)
HI *	8 (2)	0.4 ± 0.2 (0-0.6)	4.2	ND	ND	0.2	2.6	ND	3.7

ND – none detected, NA – not analyzed, * Kaneohe Bay, Oahu, Hawaii

Table 2. Comparison of mean PCB, $^{239+240}$Pu, and metal concentrations in fishes from different Pacific locations. $^{239+240}$Pu activity in muscle only from JA fish was calculated based on tissue partitioning factors and muscle mass from Noshkin (1987). Muscle activity = eviscerated fish activity X muscle partition factor (0.045 for surgeonfish and 0.075 for goatfish); Muscle mass = total fish mass X 0.663; Muscle concentration = muscle activity (pCi)/muscle mass (kg).

Location	∑PCB ng/g	As µg/g	Cd µg/g	Cr µg/g	Cu µg/g	Pb µg/g	Zn µg/g	$^{239+240}$Pu pCi/kg muscle
Johnston[*]	630.3	1.9	0.07	0.5	2.6	0.7	16.1	0.05
Hawaii[*]	-	-	-	-	-	-	-	ND
Midway[#a]	392.5	-	-	-	-	-	-	-
Tern Is.[#b,c]	12,726	25	1.0	12.2	36.8	26.6	28.0	-
Disappearing Is.[#b,c]	393	25	ND	8.0	7.8	10.5	7.0	-
Marshall Is.[*d]	-	-	-	-	-	-	-	0.1
Bikini[*e]	-	-	-	-	-	-	-	0.35

[*] wet weight, [#] dry weight, [a] Hope et al. (1997), [b]Miao et al. (2001), [c]Miao et al. (2000), [d]Robison et al. (1997), [e]Robison et al. (1997).

HO site (1) (Table 1). TCDD was only detected in one other sample (squirrelfish, *Sargocentron tiere*) from site 3. Other PCDD/PCDF congeners and dioxin-like chemicals including mono-ortho and non-ortho PCBs (Zabel et al. 1995, van den Berg et al. 1998) used to calculate TCDD toxicity equivalents (TEQ) were detected in fishes from sites adjacent to the former HO site (2,3,4) as well as East (12) and Sand Islands (8).

Fish tissue concentrations of dioxin related compounds did not exceed environmental concentrations associated with risk to aquatic life. The TEQ in fish tissue was 40.6 pg/g while concentrations associated with low risk to aquatic life is 50 pg/g (US EPA 1993).

PCBs were detected in all fish tissues, but in much higher and more variable concentrations from Sand Island (8) and Tank 49 lagoon (6), sites with known sediment PCB contamination (Table 1). Mean fish PCB concentrations at sites with no known PCB contamination ranged from 12.8 to 36.6 ng/g compared to 1,432 and 753.5 ng/g at contaminated sites. Ecological benchmarks reported for PCB concentrations in whole fish tissue are 0.1, 0.5, and 50 µg/g (Swain and Holmes 1985, Beyer et al. 1996, BCMOELP 1998). PCB concentrations ranging from 2.0 to 6.8 µg/g at site 6 and 0.3 to 3.3 µg/g at site 8 exceed the lower benchmarks.

During the 2001 sampling event all sites sampled (1,7,8,11) contained detectable metals compared to earlier sampling events where only Zn, Ba, and occasionally Hg and Cu were detected. There were no consistent site differences in metal concentrations.

Radionuclide analyses of reef fish specimens revealed that 10% of fish samples and 56% of viscera samples had detectable levels of ^{241}Am activity (Table 1). ^{238}Pu was detected in 16 and 35% of tissues and viscera. $^{239+240}$Pu activity was measured in 32 and 72% of fish tissues and viscera respectively. ^{244}Cu was only detected in one sample respectively from the RCA (5) and Sand Island (8). ^{242}Pu was detected in 6 and 12% of tissues and viscera. Radionuclide activity in reef fishes was highest offshore of the RCA (5). Fewer detections were found in Kaneohe Bay, Hawaii samples although ^{241}Am, ^{238}Pu, and ^{242}Pu activities were similar. $^{239+240}$Pu and ^{244}Cu were not detected in Hawaiian tissue or viscera (Table 2).

Discussion

The objective of this analysis was to provide a summary of the majority of contaminant data currently available for marine fishes from Johnston Atoll. With the exception of radionuclide analysis, fish whole bodies were analyzed to evaluate exposure and bioaccumulation.

Contaminants in fish tissues were greatest at sites with known sediment contamination. Compared to other contaminants, PCBs accumulated to much higher levels in fish tissues than found in sediments. Mean PCBs in sediments from site 6 and 8 were 86.6 and 35.7 ng/g compared to an average of 1432 and 735 ng/g in fish tissues (Lobel and Kerr 2002). In contrast, mean TCDD (at site 1) concentrations in tissues (5.5 pg/g TEQ) were lower than average sediment concentrations (17 pg/g TEQ). These differences may be a function of different levels of contaminant localization.

Radionuclide activity in fishes from JA was detected but low compared to locations in the Marshall Islands where extensive nuclear testing

occurred (Robinson et al. 1997). Ingestion and uptake can still occur however, since the highest radionuclide activities were found within the viscera. Noshkin (1987) suggested that plutonium uptake is lower, or that plutonium is less bioavailable to JA fish since it was produced during an aborted test and is likely a different form compared to the nuclear generated plutonium found at Bikini, Enewetak and other Marshall Island locations.

Assessing impacts of chemical contaminants in coral reef environments is difficult due to a lack of baseline monitoring criteria as well as appropriate screening guidelines for risk assessment (Jameson et al. 1998). Since tropical organisms may not respond to xenobiotics in the same manner or at the same concentrations as temperate organisms (Johannes and Betzer 1975), it is important to both quantify and monitor specific stressors and ecological responses in order to understand concentrations in the field at which adverse effects are observed (Peters et al. 1997).

These data provide baseline information for future surveys and for comparisons of chemical contaminants in the fish biota from Johnston Atoll to fishes in other coral reef habitats. An expanded analysis of this data set will examine the pattern of contamination by species and trophic guild (Lobel and Lobel ms). Long term monitoring is needed since the implications of these contaminants on the reef biota are still being evaluated.

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Ecological Effects of the Crown-of-Thorns Starfish Removal Programme on Chumbe Island Coral Park, Zanzibar, Tanzania

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Abstract. Data on reef benthos has been annually collected on Chumbe, Bawe and Changuu reefs close to Zanzibar, Stone Town, since 1992. The data shows that the El Niño in the Indian Ocean in 1997/1998 reduced the coral cover on most reefs with up to 30%. After the El Niño, a slow recovery was noticed until a major Crown-of-Thorn Starfish (COTS) outbreak occurred in 2002/2003 followed by a dramatic decrease in live *Acropora* cover. Only one of the reefs shows an exception – the protected Marine Sanctuary on the western side of Chumbe Island. When the first COTS were seen, the park management immediately initiated an on-going COTS removal programme, which has had a significant positive impact on the reef recovery. On the protected reef, the percent cover of live coral is back on the same level as before the bleaching event. Manual COTS removal programmes are often seen as a waste of time and resources, as their impact are often short term. But the results from this study argue that in a relatively small reef area it is a viable option for maintained or increased reef health and for improving recovery prospects of coral after large natural disturbances.

Key words: COTS, coral mortality, Zanzibar

Introduction

The crown-of-thorn starfish (COTS), *Acanthaster planci*, belongs to the family of *Acanthasteridae*, class *Asteroidea* (starfishes), in the phylum of *Echinodermata*. It is a coral predator causing great damage to coral reefs worldwide when in outbreak condition, e.g. Guam: Chesher 1969; Great Barrier Reef: Pearson & Endean 1969; Japan: Yamaguchi 1986). Their preferred prey is *Acropora* sp. (Endean and Cameron 1990; Pratchett 2001).

Previous records show that Crown-of-thorns-starfish outbreaks were rare events in Tanzania (UNEP 1989). The first reported COTS case occurred in 1988 and only one reef (Pange) was affected (UNEP 1989). Smaller incidences occurred in 1996/97 on Changuu reef, Zanzibar (Mohammed et al., 2000). A serious outbreak started in 2002 on a few reefs close to Zanzibar and then expanded to infest more that 60 % of Tanzania reefs by 2006 including Tanga, Zanzibar, Pemba, Songosongo, Mafia and Mnazi bay reefs (Obura et al. 2004; Mohammed et al. 2005; Obura 2005; Ussi 2008). Unlike in preceding years, the 2002-2006 outbreaks were wide spread and have persisted for a much longer period. Up to now (2008) the Crown-of-thorns-starfish threat has not completely disappeared. Repeated outbreaks have been reported in Zanzibar, Tanga and recently in Mikindani Bay, Mtwara. Such prolonged and frequent Crown-of-thorns-starfish outbreaks are unprecedented in Tanzania. It is not clear whether or not these outbreaks are linked to climate change, overfishing, eutrophication or a combination of all these factors or even just a natural fluctuation in the population.

Instead of waiting for natural processes to control the numbers of Crown-of-thorns-starfish, Chumbe Island Coral Park Ltd. (CHICOP), the managing company of the Chumbe Reef Sanctuary established in 1994, introduced a removal program in 2004 as a way of averting loss in live coral cover. The Sanctuary is relatively small, about 1 300 m long and 300 m wide and the company has trained park rangers stationed on the island at all times. Unlike in other parts of Tanzania (Tanga, Dar es Salaam, and on reefs off Zanzibar town), the removal programme in Chumbe was started immediately after the outbreak; it was consistent and long term.

The impact of Crown-of-thorns-starfish appeared to be enormous and there was an urgent need to carry out assessments more widely. Aside from their potential to cause widespread coral destruction, selective feeding by *A. planci* causes differential mortality among coral species and can exert a major influence on coral community structure (Potts 1981, Moran 1986, Birkeland & Lucas 1990, Pratchett,

2001). One way of revealing Crown-of-thorns-starfish impact is through the analysis of long term trends in live coral cover against the corresponding Crown-of-thorns-starfish outbreaks and other mortality factors. This type of data in Tanzania has been collected annually through existing coral reef monitoring programs, e.g. Scuba based coral reef monitoring at Institute of Marine Sciences (Mohammed et al. 2000, 2002; McClanahan et al., 2007a; Muhando, 2008) or Community Based coral reef monitoring such as the programme existing in Tanga (Horrill et al. 2001). Alternatively, COTS removal programmes offer an ideal scenario to reveal the impacts when sites where COTS are collected are compared with uncontrolled areas. In this study the Crown-of-thorns Starfish Removal Programme on the protected reef on Chumbe has been studied to reveal its ecological impacts on live Acroporids and non-Acroporids by comparing benthic data with data from other unprotected nearby reefs. The specific objectives of this study were: i) to analyse coral reef monitoring data collected by Institute of Marine Sciences and to establish trends in live coral cover of *Acropora* and non-*Acropora*, ii) compare coral cover changes between reefs where the population of Crown-of-thorns-starfish was controlled (individuals continuously removed) and sites where there was no removal programs.

Material and Methods

This study was conducted on three nearby (14-16 km) reefs: Chumbe, Bawe and Changuu reefs, located on the west coast of Unguja Island (Figure 1). A consistent and long term Crown-of-thorns-starfish removal program was initiated on the Chumbe Reef Sanctuary (no-take) reefs in 2004. Bawe and Changuu reefs were not protected from Crown-of-thorns-starfish impact in any way. Data on live coral cover of *Acropora* and non-*Acropora* from 1994 to 2008 was obtained from the Institute of Marine Sciences coral reef monitoring database. Reef benthos (*Acropora*, non-*Acropora*, soft corals, calcareous algae, fleshy algae, sponges, dead corals, etc) was recorded using Line-Intercept Transect (LIT) method. The density of macro-invertebrates including Crown-of-thorns-starfish were also recorded. Additional density information was obtained from other researchers in the study area (e.g., Ussi 2008). Important environmental factors likely to influence large scale coral mortality, such as SST were recorded using Tid-Bit Stowaway temperature loggers since 1997. Two major intersession events occurred: the 1998 coral bleaching event (Muhando 1999; Mohammed et al. 2000; Wilkinson et al. 1999) and the 2002-2006 Crown-of-thorns-starfish infestation (Ussi 2008). Impacts of these events on coral reefs were reviewed from various studies conducted in the study areas.

Crown-of-thorns-starfish removal on the Chumbe reef was carried out by resident park rangers while snorkelling. Chumbe reef is relatively shallow and rangers used handmade wooden sticks and recycled rice bags to carry out the removals. All removed Crown-of-thorns-starfish were counted, measured and then buried on the beach sand.

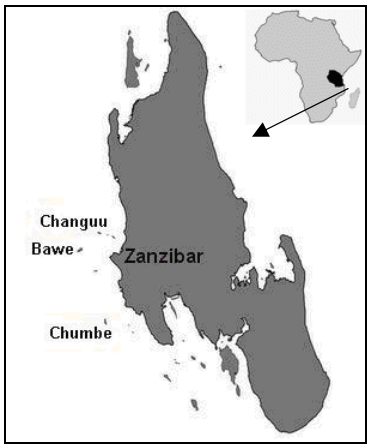

Figure 1: Map showing the study area of Zanzibar, Tanzania

Results

Coral cover

The reef benthic data shows that all studied reefs had around 50-60% total live coral cover back in the early 1990's (Figures 2a 2b and 2c). The first decrease of *Acropora* cover was noticed on Changuu in 1996 when the first isolated COTS outbreak took place (Figure 2a). Since then *Acropora* have not recovered due to long term presence of COTS and Changuu reef is now dominated by *Porites (*mostly *P. cylindrica*, *P. rus* or *Synarea rus*) and *Galaxea astreata*. Only a slight decline in *Acropora* occurred in Bawe as a result of the 1998 coral bleaching, which was mild compared to the bleaching that took place on Chumbe (Figure 2c; Muhando 1999). On Bawe, *Acropora* cover remained stable at about 21 % until Crown-of-thorns-starfish outbreak in 2003. Since then the *Acropora* cover has declined steadily down to 1-2 % in 2007 and 2008 (Figure 2b). The impact of the 1998 El Niño related increase of seawater temperatures about 2-3 ^{o}C above average (Muhando, 1999) was highest on Chumbe, where around 50% of all corals bleached and up to 90% of *Acropora* species bleached (Muhando, 1999; Mohammed et al., 2000) with total mortality of about 30% (Figure 2c). Unlike in other sites where *Acropora* abundance has substantially declined, the removal program at Chumbe prevented COTS destruction resulting in steady recovery of *Acropora* and non-*Acropora*. Figure 2d shows an

overview of the *Acropora* cover on all three reefs from 1992-2008.

Trends in non-*Acropora* cover in unprotected areas showed no significant change during both 1998 bleaching and Crown-of-thorns-starfish outbreak (Figs. 2a and 2b). Some species of *Montipora* and *Echinopora* were noticed to immediately colonise the dead *Acropora* framework, while *Porites rus* and *Galaxea astreata* and few other species were less affected by bleaching (Muhando 1999; McClanahan 2007b) and COTS predation.

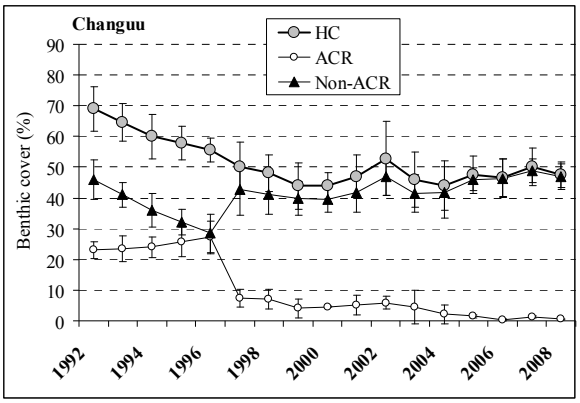

Figure 2a: Trends in live cover (%) of *Acropora* (ACR), non-*Acropora* (Non-ACR) and total live coral cover (HC) on Changuu reef from 1992 until 2008

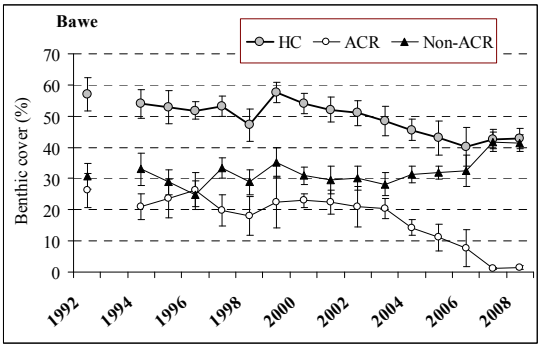

Figure 2b: Trends in live cover (%) of *Acropora* (ACR), non-*Acropora* (Non-ACR) and total coral live cover (HC) on Bawe reef from 1992 until 2008

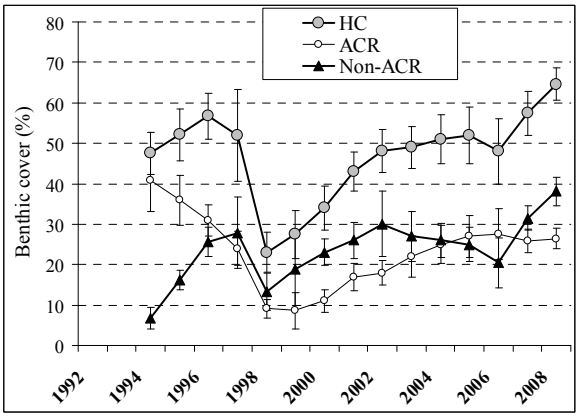

Figure 2c: Trends in live cover (%) of *Acropora* (ACR), non-*Acropora* (Non-ACR) and total live coral cover (HC) on Chumbe reef from 1994 until 2008

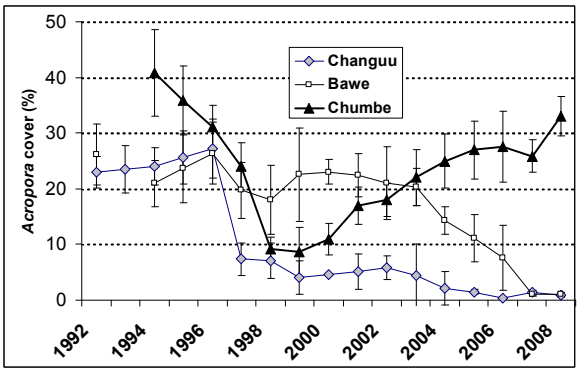

Figure 2d: An overview of the *Acropora* cover on all three reefs from 1992-2008.

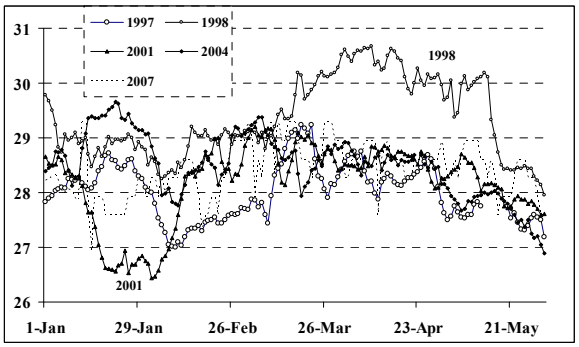

Figure 3: Daily mean seawater temperature (SST) for the months of January-May off Zanzibar town. Elevated seawater temperature in March-April 1998 was associated with coral bleaching and mortality.

Chumbe COTS removal programme

Since the programme started in 2004 a total of 118 collection days have taken place and more than 3,000 COTS have been removed inside the marine park (Figure 4), keeping the densities of COTS to almost zero at all times. Highest number of Crown-of-thorns-starfish removed per month was in January-March 2005 when 661 COTS were collected within the approximately 0.4 km^2 large MPA.

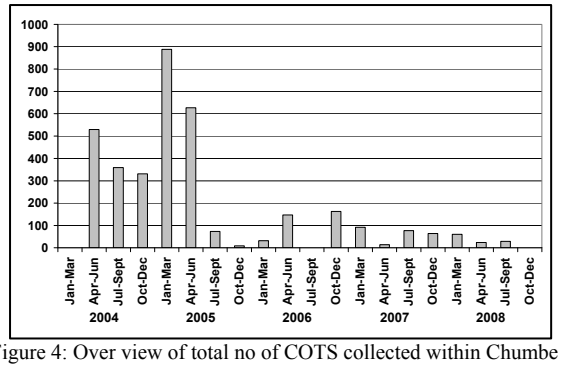

Figure 4: Over view of total no of COTS collected within Chumbe reef sanctuary since 2004

Discussion

Significant changes in *Acropora* cover were linked to the 1998 coral bleaching event (Figures 2b and 2c; Wilkinson et al., 1999) and infestation of Crown-of-thorns-starfish (Figure 2b). This study confirms that the 1998 coral bleaching event and the outbreak of Crown-of-thorns-starfish had more negative impact on *Acropora* when compared with non-*Acropora*. Regeneration of *Acropora* after the bleaching event was halted by outbreak of Crown-of-thorns-starfish, among other degradation factors in unprotected reef areas, while it flourished well in Chumbe where the removal program kept the Crown-of-thorns-starfish density on a low, in addition to other management actions. On the other hand, the removal program didn't have obvious impact on non-*Acropora* corals, at least in the studied areas.

Long term coral reef monitoring data can be useful in linking causes and impacts with natural and human induced disasters on coral reefs. In this study fast decline in *Acropora* was linked to coral bleaching event and Crown-of-thorns-starfish outbreaks. However, non-*Acropora* (as a group) did not show clear links. Such selective mortality has already been demonstrated elsewhere, e.g., McClanahan et al., (2007b) for bleaching and Pratchett (2001) for COTS. *Acropora* seem to be defenceless, easily accessible, palatable, high nutritional value, convenient structure (firm grip against wave actions) and few aggressive/defensive symbionts (Endean and Cameron 1990; Pratchett 2001) and all of the above mentioned features make *Acropora* an ideal target for COTS predation.

When compared to previous outbreaks in 1988 (UNEP 1989) and 1997 (Mohammed et al. 2000), the recent Crown-of-thorns-starfish population outbreak was unprecedented widespread and persisted for longer period. Its persistence and duration appears to suggest a significant environmental change towards favourable conditions for COTS. It is not clear at this stage whether or not this is an indirect impact of climate change (global warming).

Crown-of-thorns-starfish removal program has demonstrated that exclusion of predators can be an effective strategy in enhancing recovery after natural disasters (e.g., El Niño). Unlike in Bawe and Changuu reefs where no clean up action took place, the demise of *Acropora* in Chumbe was rescued. On the other hand, results suggest that non-*Acropora* corals were much more stable. Such differential coral mortality caused by selective feeding habits has a direct influence on reef structure (McClanahan et al. 2007b). In the unprotected study areas the coral composition has changed in favour of *Porites rus* (Synarea), branching *Porites* (*P. cylindrica*) and *Galaxea astreata*. High abundance of Corallimorpharia was noticed on the reef flat and reef slope, covering the dead *Acropora* plates and rubbles (Muhando et al., 2002). *Acropora* is no longer the dominant coral genera today in many unprotected reefs, e.g. Bawe and Changuu. Reef fish that depend directly on *Acropora* for their replenishment are likely to decline as a result (Garpe et al. 2006). The impact of these changes to ecosystem functions requires special research attention.

This study has demonstrated the need to encourage coral predator removal programs, specifically Crown-of-thorns-starfish, as one of the management options to achieve increased coral cover. The removal methodology used was inexpensive and convenient only for shallow water reefs. The use of SCUBA divers is however necessary in case of deeper reefs, though it will increase operation costs. Part of Chumbe success was the decision to initiate the removal at early stages of outbreak along with the continuous monitoring of the reef. Removal programs in Dar es Salaam (Wagner 2004) and in Tanga were not successful because COTS removal was initiated well after significant damage to coral ecosystems occurred. Thus, COTS management requires effective institutional (approving and sponsoring) mechanisms to be in place for making prompt decisions regarding removal programs. Unlike in unprotected areas, the overall management actions within the MPAs also contributes to improved reef health and productivity in the face of human impacts (Russ et al. 2003; McClanahan and Mangi 2000)

Prediction knowledge of when and where *A. planci* outbreaks will occur is still lacking. This is essential to improve future control and increase the chances of success from removal programs. Furthermore, research is also needed to determine factors influencing dynamics of Crown-of-thorns-starfish. For example, a hydrodynamics studies found that due to the interaction of tidal, gravity and wind flows, some areas within the reef retained higher numbers of larvae than others (Black and Moran 1991). Surveillance of these locations, which may be correlated with initial recruitment of *A. planci*, could provide an 'early warning' strategy for monitoring and controlling future outbreaks of this starfish on reefs (Black and Moran 1991). The main long term objective of research at this stage in the western Indian Ocean, is to find out what factors and processes that favour these out-breaks of COTS populations so they can be dealt with and the problem can be avoided before it even occurs.

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Empowering fisher women through ICT in reef conservation and management – a case study from Tuticorin coast of the Gulf of Mannar, Southeastern India

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Abstract. To reduce pressure on coral reef resources and economic vulnerability of coastal communities, local fisher women Self Help Groups (SHGs) were empowered through provision of Information and Communication Technologies (ICT) and adult education in 5 coastal villages in Tuticorin district of the Gulf of Mannar (GoM) in South-eastern India. Improved literacy levels, environmental education, as well as the provision of computer training and equipment, enhanced villagers ability to take up alternative livelihoods and improve their living conditions. The support to SHGs demonstrated their potential as a non-threatening mechanism for mobilizing resources, providing affordable finance and social benefits to poorer fisher women, besides promoting self-reliance, awareness creation, capacity development, social solidarity and the empowerment. Village coordinators from five targeted villages were trained and each village was provided with computer, printer, mobile phone, and internet. In addition, the SHG members in the targeted villages were also trained in other alternative livelihood activities such as vermi-compositing and hygienic fish drying methods. The creation of awareness about the environment along with the adult education, computer training and other livelihood options helped the fisher women to earn additional income for their families, the key factor in to reducing the destructive fishing practices and enhancing living conditions in the coastal areas of GoM.

Key words: Reef management, adult education, alternative livelihood, computer education

Introduction

Information and Communication Technology (ICT) is developing fast in India. Government has elevated the livelihood of village folk through a system called Self Help Groups (SHGs) in their respective villages. The SHGs play a major role in the generation, saving and wise use of financial resources. The majority of the SHGs are formed by women and Government encourages SHGs to create confidence particularly among the women. The support to SHGs with ICT can empower them in environmental education (literacy), livelihood options (for socio-economic development), marketing strategies, information on natural disasters and concern about conservation and management of natural resources. Suganthi Devadason Marine Research Institute (SDMRI) in collaboration with Coastal Ocean Research and Development in the Indian Ocean (CORDIO) in East Africa, and Nyköpings Folkhögskola in Sweden initiated the ICT based activities among fisherwomen Self Help Groups in India. The adult education concept has also been tried in other parts of the world as a contributor to national development in Southern Africa (Oduaran and Okukpon 2005); for healthy participative democracy in Scotland (Hammond 2006); and in adult Education and Training in Ireland (Morrissey and McNamara 2004). The aim of introducing the present adult education along with the provision of Information and Communication Technologies in the 5 coastal villages in Tuticorin district of the Gulf of Mannar in the Southeastern India was: 1) to empower local fisherwomen Self Help Groups (SHGs) 2) enhance literacy and livelihood 3) reducing pressure on coral reef resources through greater awareness and education about marine environment and resources 4) minimise overall economic vulnerability of coastal communities.

Methods

Five villages namely, Siluvaipatti, Rajapalayam, Arockiyapuram, Thirespuram and Inigo Nagar were selected in Tuticorin coast of the Gulf of Mannar, Southeastern India. Two coordinators (SHG members) from each village were selected. The coordinators were given training in adult and environmental education and computer applications. After the training, each village was provided with ICT components (including computer, printer, mobile phone, and internet). The coordinators then trained their respective villagers in adult and environmental education; and computer training. The villagers were also given training in alternative/additional livelihood schemes such as vermi-composting and a hygienic seafood drying method. Baseline information on literacy,

occupation, awareness about corals, coral status, destructive fishing practices etc was collected before the start of the activities.

Results

During Oct 2007-Feb 2008, 149 fisherwomen and 6 -men in five villages participated in adult and environmental education was, 34 women and 48 school children in computer education; and 5 women were trained in vermi-composting and 37 in hygienic fish drying.

Participants learned to write their names instead of signing by thumb print; started to read Tamil (local language) and English words; those who already knew to write their names in Tamil learned to write it in English; learned to read bus boards and started to travel alone; learned simple mathematical calculations during adult education classes; women participants are helping their children in their studies; and after witnessing the improvement, more coastal women are willing to take part in the adult education.

The participants of the adult education were taught about cleanliness in their environment and the importance of conservation of natural resources, particularly corals. Increased level of awareness were tested through quizzes and tests. Participants were also made aware of the importance of corals and a clean coastal environment as well as the need for their conservation by way of demonstrating eco-friendly fishing practices, by avoiding disposal of untreated sewage, industrial waste and dumping of solid waste on the coasts. Also information about global warming and its effects on corals was given through video clips and charts.

The programme included group discussion and entertainments (songs, folk dances, drama). The coordinators also participated in the awareness campaign through the National Broadcasting Corporation. Also, women and children received computer classes that met with much success.

Women were taught hygienic ways of sun drying of fishes using indigenous fish drying racks to enhance the quality and price of the dried product. The women in four villages now exclusively use the fish drying racks and the fish, free of dust and sand particles, fetch a better price. In addition, less drying time is required when of drying on the floor.

Village coordinators were trained in vermi-composting and quickly started earning by selling the product in their villages.

The awareness campaign in the five villages bordering Gulf of Mannar had resounding success and resulted in considerably reduced damage on reefs. Shore seine operations, mining and anchoring near the reefs declined. New coral recruits were observed and the live coral cover area is increasing in the once heavily degraded reef areas.

Conclusion

The coral reefs in the Gulf of Mannar are facing various anthropogenic and natural threats but in Tuticorin, several village communities are solely dependent on fish resources obtained from these coral reefs (Shanthini et al. 2002). Crowded fishing grounds, increasing demand for fisheries products, and declining catches often cause fishermen to adopt destructive fishing methods (Samuel et al. 2002) for better results. To counter this trend, the ongoing community based initiative helped to increase literacy level, technical capability in ICT and alternate livelihood practices and knowledge of resource conservation especially eco-friendly fishing, particularly in the reef areas. Fisher folk responded positively to the training initiative and enforcing reef protection has also become easier due to the greater awareness about corals and conservation and environmental education among the villagers.

The creation of environmental awareness along with the adult and environment education, training in computer applications and other livelihood options helped the fisher women to earn additional income for their families, which is key to reduce the destructive fishing practices and enhance living conditions in the coastal areas of the Gulf of Mannar. This pilot effort helped the fisher folk to change attitudes resulting in considerable reduction in disturbance to reefs and surrounding habitats as evidenced by new coral recruits and increased live coral cover.

Acknowledgement

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Habitat similarity between an artificial reef and the surrounding natural hardbottom in Broward County, FL, USA

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Abstract: In order to mitigate for anticipated burial of natural hardbottom due to the Broward County Shoreline Protection Project, 3.6 x 10^{-2} km^2 (8.9 acres) of mitigative artificial reef were constructed in August/September 2003. To determine if the artificial reef was approaching community similarity to the surrounding nearshore natural hardbottom, benthic biological monitoring was conducted over five years at six discrete monitoring events. Twenty-seven, 30-meter transects were monitored on the artificial reef and 26, 30-meter transects were monitored on the natural hardbottom. Benthic monitoring utilized the Benthic Ecological Assessment of Marginal Reefs (BEAMR) methodology. The faunal and floral benthic community remained significantly distinct between the artificial and natural reefs five years post-mitigation; however similarity has increased notably over time. Changes in the biotic benthic community structure have slowed on the artificial reef and may be reaching stabilization. The ultimate limiting factor in community similarity between the artificial reef and the surrounding natural hardbottom may be attributed to the differences in structural complexity.

Key words: artificial reef, natural hardbottom, similarity, functional groups, benthic community

Introduction

Ecologically, there is an expectation that an artificial reef will resemble or exceed local natural environments over the long-term as assemblages associate with its surface, structure, and surrounding water column (Seaman and Jensen 2000). Long-term studies are essential to determine possible differences between artificial and natural reef communities.

Common initial settlers of artificial reef such as hydrozoans, polychaetes, mollusks, bryozoans, tunicates, and sponges constitute a 'minor' benthic component (percent cover) but are important in elevating the structural complexity of the artificial reef substratum, eventually establishing an area for coral settlement (Perkol-Finkel and Benayahu 2005). Connell and Keough (1985) found that recruitment of benthic flora and fauna onto an artificial reef occurs primarily by migration from adjacent substrate and by the settlement of spores and larvae. Physical factors such as currents, water depth, and the quality of the substrate influence the recruitment of benthic species onto the mitigation reef. Young (1995) suggested that the introduction of new substrate contributes to an increase in epibenthic organisms which, in turn, provide a larger quantity of food.

Due to the anticipated impact to natural hardbottom areas during beach renourishment activities in Segment III of the Broward County Shore Protection Project (SPP), the Florida Department of Environmental Protection (FDEP) required the placement of 3.60 x 10^{-2} km^{-2} (8.9 acres) of mitigative artificial reef in the nearshore environment. Segment III is approximately 10.97 km (6.8 miles) in length and is located between Port Everglades and the Broward/Miami-Dade County line in south Florida (Fig. 1).

The mitigative artificial reef was constructed as one layer of limestone boulders (1.2 - 1.8 m maximum diameter) placed in the nearshore zone in approximately 4 to 6 m mean water depths. The mitigation reef was constructed in six discrete areas between FDEP survey monuments R-101 and R-125 during August and September 2003.

The anticipated burial of natural hardbottom initiated a comparative evaluation between natural hardbottom areas and the mitigation reef. The monitoring program intended to measure the recovery potential that mitigative reefs provide to compensate for the anticipated burial of natural hardbottom.

Methods

Benthic assessment

Twenty-seven fixed 30-meter transects were monitored on the artificial reef and twenty-six fixed 30-meter transects were monitored on the natural hardbottom for comparison. Monitoring took place at six discrete sampling events between May 2004 and September

2008, and are referred to as the 9-, 12-, 18-, 24-, 36-, and 60-month post-mitigation monitoring events.

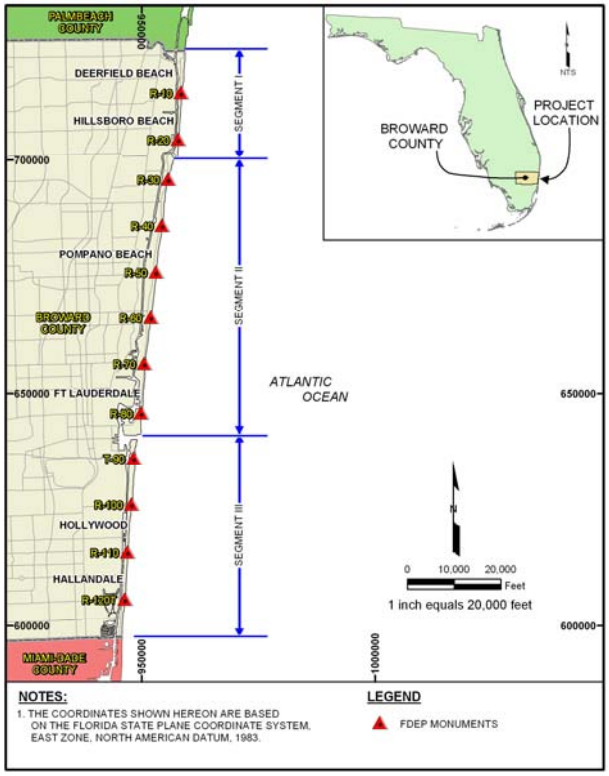

Figure 1: Location map of Broward County Segment III Shore Protection Project.

Benthic communities were evaluated using the Coastal Planning & Engineering, Inc. Benthic Environmental Assessment for Marginal Reef (BEAMR) method (Lybolt and Baron 2006) which uses percent cover as a proxy for abundance. BEAMR was conducted at each specified transect using 12 replicate 1.0-m^2 quadrats (1.0 m x 1.0 m) every 2.5 meters, starting at 0.0 m for a total of 12 m^2 planar area per transect. The 9-month post-mitigation BEAMR surveys were conducted using 0.25-m^2 quadrats (0.5 m x 0.5 m). The quadrat size was changed to 1.0-m^2 in order to align these data with the Shore Protection Project beach construction compliance *in situ* monitoring, a parallel project. All subsequent surveys used the 1.0-m^2 quadrats.

Statistical analysis

PRIMER v6 non-parametric statistical package (Clarke and Warwick 2001) was used to compare the benthic assemblage between the artificial reef and the adjacent natural hardbottom using transect-level average percent cover for functional groups and macroalgae genera. The data were standardized by total and transformed as appropriate. Similarity Percentages (SIMPER) were applied to determine which taxa contributed to the average dissimilarity ($\bar{\delta}$) between substrate types (artificial vs. natural). A Bray-Curtis similarity matrix was applied to the transformed dataset to generate multi-dimensional scaling (MDS) ordinations and analysis of similarity (ANOSIM) routines in order to determine changes in the level of similarity between the artificial reef and natural hardbottom over time.

Results

Functional Groups

Natural Hardbottom vs. Artificial Reef

Sediment is one of the functional groups sampled for in BEAMR but was removed from the dataset in order to compare the biotic benthic community between the two reef types. Table 1 presents percent contribution of each taxa to the average dissimilarity between the artificial and natural reefs with a cutoff of 70%. Macroalgae, zoanthid, and turf algae cover generally dominated the contribution to the average dissimilarity. By 60-month post-mitigation, the dissimilarity decreased to 25.54% between the reef types.

Table 1: The percent contribution of specific functional groups to the average dissimilarity between the artificial reef and natural hardbottom.

Functional Group	Percent contribution of the i^{th} taxa to the average dissimilarity b/n the artificial reef and natural hardbottom					
	9-Mo	12-Mo	18-Mo	24-Mo	36-Mo	60-Mo
Macroalgae	15.90%	11.70%	11.71%	7.38%	11.59%	12.45%
Zoanthid	11.51%	14.04%	14.12%	14.38%	11.90%	13.00%
Turf algae	13.85%	8.97%		8.46%	12.32%	
Sponge	8.48%		5.97%		9.61%	11.99%
BHS	7.78%	10.90%	11.43%	21.77%	5.66%	
Octocoral		11.10%	9.25%	9.67%	11.64%	7.32%
Wormrock			11.58%	9.72%		5.87%
Bryozoan	10.19%					
Stony coral	7.44%	8.84%	8.38%		4.43%	
Encr red algae		6.93%				
Tunicate					5.14%	6.16%
Bivalve						8.27%
Hydroid						5.58%
Average dissimilarity ($\bar{\delta}$)	33.34%	34.27%	30.97%	41.88%	33.14%	25.54%

*BHS is bare hard substrate

Abiotic and floral groups were removed from the dataset to compare the faunal functional group assemblage between the artificial reef and natural hardbottom at each discrete monitoring event. MDS ordinations were generated and SIMPER and ANOSIM routines were applied to determine the average dissimilarity and the degree of similarity (R-value) between the two substrate types (Fig. 2).

Artificial Reef over Time

The biotic benthic community of the artificial reef was plotted in an MDS ordination to display change in community composition over time. Fig. 3 indicates

progression towards a stabilizing community as the most recent datapoints cluster together. A post-hoc ANOSIM supports this hypothesis, where the lowest R-values are found between 18 and 60 months post-mitigation (Table 2).

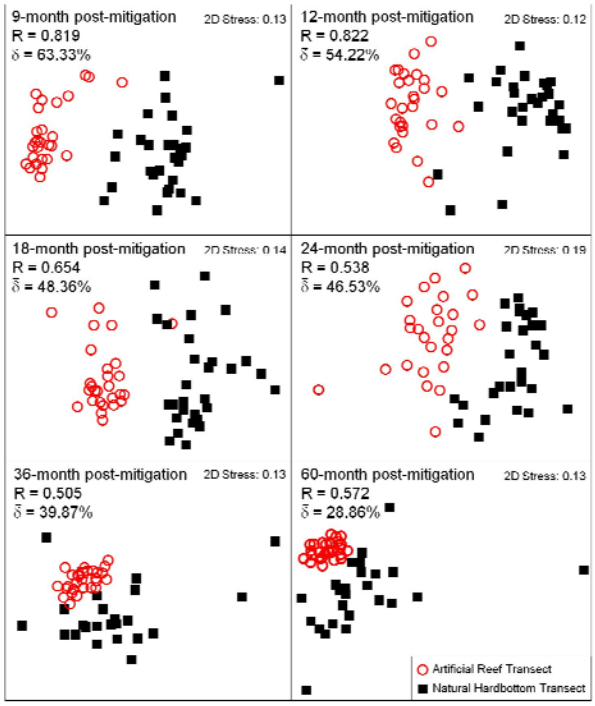

Figure 2: MDS plots of the artificial reef and natural hardbottom benthic faunal assemblages with associated ANOSIM and SIMPER results (R and $\bar{\delta}$).

Macroalgae

Macroalgal recruitment and community development was of particular concern on the artificial reef due to the anticipated loss of turtle foraging habitat from hardbottom burial. Makowski et al (2006) and Wershoven and Wershoven (1992) identified 12 genera of macroalgae that are common to the diet of juvenile green sea turtles (*Chelonia mydas*) in Palm Beach and Broward Counties. Six of the seven 'preferred' macroalgae that are found on the natural hardbottom were identified on the artificial reef.

In total thirty-four genera of macroalgae were identified on the artificial and natural substrates. Thirty-two were found on the natural hardbottom and 25 were identified on the artificial reef. Fig. 4 presents the average percent cover of macroalgae on the artificial and natural substrates as well as the average dissimilarity (determined from SIMPER) between the two substrate types at each monitoring event.

Macroalgae cover increased steadily with an associated decrease in dissimilarity, until a sharp decline after the 18-month monitoring event. This is likely a result of the intense hurricane activity experienced in south Florida in 2005, which may have caused scouring on both substrate types. Macroalgae cover recovered and continued to increase on the artificial reef by 36 months but remained relatively low on the natural hardbottom. As cover on both substrate types increased, dissimilarity decreased and vice-versa.

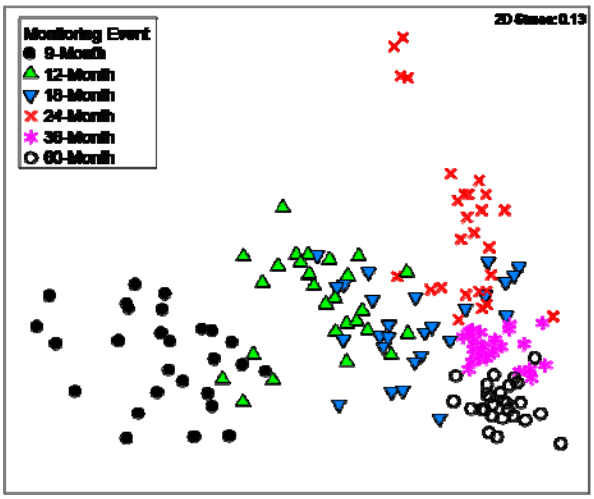

Figure 3: MDS plot of the biotic benthic community on the artificial reef over time.

Table 2: R-values from ANOSIM comparing the similarity in the biotic benthic community on the artificial reef over time.

ANOSIM R-value	9-Mo	12-Mo	18-Mo	24-Mo	36-Mo
12-Mo	0.688				
18-Mo	0.954	0.448			
24-Mo	0.981	0.731	0.520		
36-Mo	0.999	0.872	0.537	0.636	
60-Mo	0.999	0.928	0.795	0.733	0.670

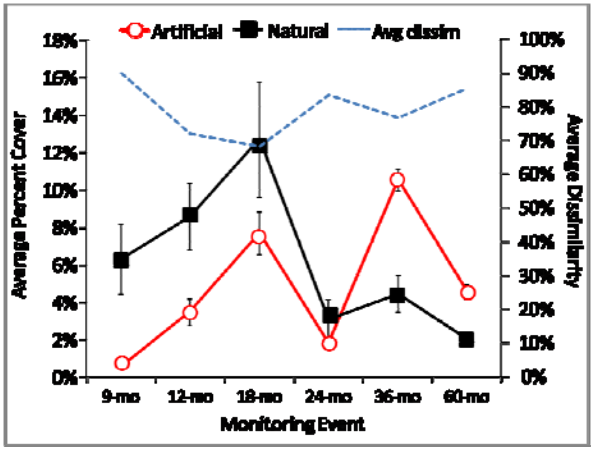

Figure 4: Average percent cover of macroalgae and average dissimilarity between the artificial and natural substrates over time.

Discussion

Connel and Keough (1985) surmise that assemblages on artificial reefs may through time converge towards

an epibiotic assemblage on natural substrata. This study set out to determine if the benthic assemblage on the Broward County artificial reef is approaching habitat equivalency, or at least similarity, to the surrounding natural hardbottom using a long-term monitoring program.

The benthic community between the two reef types proved to increase in similarity but remained functionally distinct 5 years post-deployment of the artificial reef. The dissimilarity between the macroalgal community on the artificial and natural substrates fluctuated over time displaying no obvious trend except that the factors which influence macroalgae cover appear to affect both substrates concurrently.

The dynamic assemblages that are well known on natural hardbottom off the coast of Broward County may be markedly different from the developing assemblages found on the mitigative substrate due, in large part, to variations in the biological and physical environment created by the boulders. Structural differences between artificial and natural substrates can ultimately lead to two distinct reef communities (Perkol-Finkel and Benayahu 2007). The high relief of the artificial reef boulders in Broward County will likely be the limiting factor in reaching habitat equivalency to the surrounding natural hardbottom.

Acknowledgement
We would like to thank the Marine Science and Biological Research Department at Coastal Planning & Engineering, Inc. for the monitoring program development, data collection, research and analytical support. We would also like to thank Broward County Environmental Protection Department for their participation, as this data was collected as part of the ongoing biological monitoring program for the Broward County SPP.

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St. Croix East End Marine Park: The U.S. Virgin Islands' first territorial marine park

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Abstract. Established on January 15, 2003, the St. Croix East End Marine Park (STXEEMP) is the U.S. Virgin Islands' first marine park and represents the culmination of three years of collaboration between the federal and local government, non-governmental organizations and other stakeholders. The STXEEMP is on the eastern end of St. Croix extending from the high tide boundary to the three nautical mile territorial waters limit. The park was designed to protect the largest island barrier reef system in the Caribbean and to promote sustainability of coral reefs, seagrass beds and mangrove habitats and to preserve significant natural areas for the benefit of future generations. A park managed monitoring program designed to collect scientific information to assist in the wise management of these habitats and species to ensure that these marine resources are available in the future has yet to be implemented. The park's education and outreach program's goals are to facilitate environmental awareness opportunities for the community, encourage a sense of user stewardship regarding the marine environment and promote the awareness of and support for the park. In October 2005, the park's headquarters was finally established on a historic plantation site adjacent to the park and an interpretive program implemented.

Key words: Marine Protected Area, Caribbean, regulations

Introduction

The St. Croix East End Marine Park (STXEEMP) is located at the east end of St. Croix in the United States Virgin Islands (VI) (Fig. 1). The VI consists of three large islands – St. Croix, St. Thomas, and St. John and over 50 small islets and cays. Located at the eastern end of the Greater Antilles, the VI is home to over 120,000 people and hosts over 1.3 million visitors annually. St. Croix is the largest island with a land mass of >218 km^2.

The park surrounds the east end of St. Croix from the high tide mark out to three nautical miles. The northwest boundary starts at Chenay Bay ($17^o45'39"N$; $64^o40'5"W$) and ends at the south west boundary at Great Pond Bay ($17^o42'51"N$, $64^o39'52"W$) encompassing an area of approximately 60 $miles^2$ (155 km^2) (Fig. 1). The shoreline that borders the park is approximately 17 miles (27 km) long. The park is within the jurisdiction of the VI territorial government as it lies within the three nautical mile territorial boundary. The Buck Island Reef National Monument is juxtaposed to the park and is under the jurisdiction of the U.S. Federal government. The land bordering the park is within the Coastal Zone First Tier and any development activity is subject to approval by the VI Coastal Zone Commission.

In 1960, the Department of Interior completed a study for the Governor of the VI that recommended that the east end of St. Croix be designated as a nature preserve. A series of similar designations have been made in the past 40 years for the land and waters of the east end of St. Croix, including:

- Designation as an Area of Particular Concern (APC) – 1979
- Designation as an Area for Preservation and Restoration (Teytaud 1980)
- Nomination as a Significant Natural Area (Teytaud 1980)
- Candidate for park within VI Territorial Park System plan (Alexander 1981)
- Nomination as a candidate for National Marine Sanctuary status- 1982
- Recommended as a multi-purpose park within proposed Territorial Park System (Island Resources Foundation 1991)

Management Approaches

Multi use design

In order to establish a functional multi use park a management plan was devised with four managed areas: Recreation, Turtle Wildlife Preserve, No Take and Open Fishing (Fig 1). The Recreation Areas include 2.8% of the park and are designated for snorkeling, diving, recreational fishing, bait fishing and boating while preserving resources. The Turtle Wildlife Preserve Area covers 7.0% of park waters and protects turtle nesting beaches for Green, Hawks-

bill and Leatherback turtles. The No Take Areas only encompass 8.6% of the park and are designed to protect spawning, nursery, and residence habitat for various species. The Open Fishing Areas comprise 81.6% of the park and presently provide little additional protection than exists in other territorial waters. Existing territorial restrictions govern the use of these areas; the only marine park restriction is the prohibition of the removal of coral or live rock in these areas.

No Take Areas were designed to encompass large, contiguous diverse habitats. They are intended:

- to provide natural spawning, nursery, and permanent residence areas for the replenishment and genetic protection of marine life, and
- to protect and preserve all habitats and species; particularly those not protected by fisheries management regulations.

This zone was intended to protect areas that represent the full range of diversity of resources and habitats found throughout the park.

Turtle Wildlife Preserve Areas were established:

- to minimize disturbance to sensitive wildlife populations and their habitats,
- to ensure protection and preservation of wildlife resources in the park.

This designation will be applied to the primary turtle nesting beaches and near shore resting areas. Regulations governing access are designed to protect the endangered turtles and their habitat, while providing opportunities for public use.

Recreational Areas are designed to provide areas for snorkeling, diving, and boating while prohibiting any activities that would compromise the recreational values for which the area may be designated. Restricted activities will be defined in future public input meetings. Specified recreational opportunities may be protected, enhanced or restricted, while preserving basic resource values of the area. No other uses are specifically restricted with the exception of general shipping.

Open Fishing Areas are areas in which there are no additional restrictions on fishing, boating, and diving activities. These areas are governed by all the rules and regulations pertaining to commercial and recreational fishing in the VI Code. These areas are designated to monitor and evaluate the effects of resource zoning in the park. Trawling and general shipping are prohibited, as well as those activities inconsistent with the park's long-term conservation (*e.g.*, mining and oil drilling).

Rules and regulations

Although the STXEEMP was established in January 2003, the enabling documents establishing the Rules and Regulations (R&R) were not signed by Governor John P deJongh until September 2007. The purpose of the R&R was to provide specific rules to protect park resources from both direct and indirect threats. These regulations focus on habitat protection, reducing threats to water quality, and minimizing human impact to delicate resources. The park regulations have been established to complement those in existing management areas, including Buck Island National Monument and Green Cay National Wildlife Refuge.

The following activities are prohibited park wide:

- removing, injuring, or possessing coral or live rock,
- discharging or depositing trash or other pollutants,
- dredging, drilling, prop dredging, or otherwise altering the seabed, or placing or abandoning any structure on the seabed,
- operating a vessel in such a manner as to strike or otherwise injure coral, seagrass, or other organisms attached to the seabed, or cause prop scarring,
- having a vessel anchored in a manner other than the use of a reef mooring buoy,
- operating a vessel at more than idle sped/no wake within 100 yards of park vessels and navigational aids marking reefs,
- operating a vessel at more than idle speed/no wake within 100 feet of a diver down flag,
- diving or snorkeling without a dive flag,
- operating a vessel in such a manner which endangers life, limb, marine resource or property,
- releasing exotic species,
- damaging or removing markers, mooring buoys, scientific equipment, boundary buoys, and trap buoys,
- moving, removing, injuring, or possessing historical resources,
- taking or possessing protected wildlife,
- using or possessing explosives, and
- using firearms.

In addition to the park wide rules, the following activities are prohibited in the No Take Area:

- discharging any matter except cooling or engine exhaust,
- fishing by any means, removing, harvesting or possessing any marine life,
- touching standing on living or dead coral, and
- anchoring on living or dead coral, or any attached organism.

In addition to the park wide rules, the following public access restrictions in the Wildlife Preservation Area include:

- idle speed only - no wake,
- no access buffer,

- no outboard motors, and
- establishment of limited closure periods.

The R&R do not go into effect until 30 days after public notices have been distributed stating that the park's boundary markers have been deployed. Although funds have been available for several funding cycles and the previous Department of Planning and Natural Resources (DPNR) Commissioner signed a contract to deploy the boundary bouys, the contract has lapsed. In August 2008, the ex Commissioner was sentenced to a prison term of seven years on corruption charges and misuse of Coastal Zone Management funds. As of July 2008, there was no contract for deploying the boundary markers and thereby turning the existing paper park rules and regulation into a legally enforceable code.

Enforcement strategies

The primary law enforcement objective in the park is to achieve resource protection by gaining compliance with the Park regulations and other Federal and Territorial statutes that apply within the STXEEMP. An enforcement program is one of the tools available to managers of marine protected areas, and is considered by the initial planners to be a Best Management Practice. They thought that such a program could compliment other management programs, such as research and education, and lead to increased levels of success. Of course successful enforcement would require resource managers to commit to enforcement programs that are properly supervised and funded. In the first five years of the park's existence this has not happened. The entire idea may be unrealistic in the near future given the existing political conflicts in managing the park.

The enforcement philosophy should be that preventive enforcement is best achieved by maintaining sufficient patrol presence within the Park to deter violations and by preventing, through education, inadvertent violations of the law. Successful enforcement relies on frequent water patrols, and routine vessel boardings and inspections. Water patrols would ensure that park users were familiar with park regulations, deter willful or inadvertent violations of the law, and provide quick response to violations and/or emergencies.

The success of park enforcement will depend on how well the enforcement entities on St. Croix are coordinated. Because of limited resources at the Federal, Territorial and park level, enforcement assets must be targeted and used in an efficient and directed effort to achieve compliance with existing and proposed regulations. The coordination of enforcement assets will be an integral component of the management of the park. Interagency agreements among other enforcement entities on St. Croix should be developed, including the National Park Service, US Fish and Wildlife Service, US Coast Guard, VI Department of Planning and Natural Resources (Enforcement Division) and the VI Police Dept. All of this is predicated on having senior managers in DPNR who are knowledgeable about environmental management and have the political interest and stamina to advance the park.

Monitoring and measuring success

Site evaluation and monitoring should be a continuous process, with regular reporting intervals and a formal evaluation mechanism. All monitoring plans should include acceptable limits of change. A monitoring program should be able to provide managers with fundamental information with which to make decisions, and would facilitate a flexible approach, as well as a responsive management system. A comprehensive review by a scientifically qualified review team, performed on at least a bi-annual basis, would help to ensure that implementation was occurring as planned. Site monitoring activities should have been guided by the following objectives:

- Establish a baseline within the respective use-zones within the park, thus providing a means for measuring success in the future,
- Collect park utilization data to be part of a social and cultural analyses and used to modify and enhance park regulations and activities,
- Collect biological data that are representative of the status and health of marine organisms and their respective habitats, and
- Collect fisheries data that quantify fishing trends (*i.e.*, fishing methods, species caught, amount caught, etc.) within park boundaries.

The park has yet to establish a baseline monitoring program of biophysical parameters necessary to assess climate change (Quinn & Kojis 1995) or a baseline survey of fauna and flora. Instead of hiring and training technically competent personnel the park has relied on non government organizations, the local university and federal agencies to gather data in the shallow water communities. Surveys in the deep water habitats that require equipment and skills beyond the capacity of the territorial government have not been conducted and would be an appropriate task for federal agencies.

The use of non VI government agencies is clearly an easy approach, but will later be subject to closer scrutiny as the validity of park management issues come under question. NGOs are not objective local stakeholders, but rather entities with a political agenda to advance environmental conservation supported by an off island constituency. Likewise federal agencies are viewed with suspicion and by some islanders who view federal involvement in island

resource management as meddling by outsiders. One has to wonder why support for capacity building and empowerment of territorial government agencies was not given greater priority. Certainly the objectivity of the data collected by NGOs should be subject to scrutiny. It is a rather politically dubious situation when the park bases it scientific management on the findings of an organization with a clear bias. The evidence suggests that the funding agencies have little sensitivity to the political nature of marine resource utilization in St. Croix and the importance of the capacity building of objective professional standards in territorial government agencies.

Education and outreach

Throughout the planning process, the need for a comprehensive and effective educational program was emphasized. Activities such as public forums, printed materials, public events, school tours and an interpretive program will help to advance the community's awareness of the importance and activities in the park. In January 2008, the park's interpretive program commenced with weekly tours of the watershed adjacent to the park and fauna and flora tours to schools groups of Great Pond Bay. The tours were well attended by the public and the park received favorable TV and print publicity.

Park headquarters at Estate Great Pond (EGP)

The headquarters of the STXEEMP was relocated to a restored colonial building at EGP in October 2007, almost five years after the creation of the park. The cultural resources on EGP represent a historical chronology of over 600 years of changing land uses and human occupants. During the era of Danish sovereignty (1735-1917), the Great Pond area fell within the boundaries of different plantation grounds. Due to the constant change of property ownership and land uses, the Great Pond plantations were consolidated or sold separately from 1760-1916.

Estate Great Pond is a testimony to the history of agriculture on this southern section of St. Croix. The property presently consists of a late 18th century great house, remains of a factory, slave quarters, stable area or storage room, and other historical ruins. Cotton was planted during the initial development of the plantation in the 1770's because cotton grew well in a dry environment. The 18th century storage building west of the great house and north of the factory was where cotton was stored and processed.

By the late 1700's to the early 1800's, the estate was a sugar plantation with many owners. The only documented occupation after 1800 occurred in 1843 to 1845. However, no evidence has been found of post-emancipation use by renters and/or small holders. Although, it is possible that such marginal activity may have escaped the attention of the record keepers. By the early 1900's, the Great Pond area was a sugar plantation and used for grazing animals. In 1902, the Danish West Indies' Plantation Co. purchased EGP. The area was used as an agricultural experiment station for a short time.

When the Danish Virgin Islands colony was purchased in 1917 by the United States, the EGP was gradually subdivided. Sugar production on St. Croix began to fall again after the United States purchased the islands. By 1933, much of the sugar cane producing land on the island was abandoned and the lands reverted to cattle grazing. The division of rural land including the Great Pond area by end of the decade was 75% pastures, 15% cane and 10% bush land. By the 1960's, agriculture in the Great Pond area had ceased and only cattle grazing remained.

The EGP is also identified as a Pre-Columbian site. The site stretched for more than a half mile along the coast, but generally not further than a few hundred feet from the shoreline. The first excavations conducted at the Great Pond site found substantial artifacts dating to the Longford Stage (Saladoid, A.D. 300-700) and remains from the Richmond Stage (Ostionoid, A.D. 700 - 900).

The area is registered and listed in the National Register for Historical Places. According to archaeologists, the whole site possesses considerable historic archaeological findings, and therefore needs to be protected from unnecessary ground disturbances. The history of the area includes Africans, Europeans, and native North American Indians. Therefore, the Great Pond area is very significant to the people of the VI and visitors.

In the early 1970's, the Department of Conservation and Cultural Affairs recognized the need to provide recreational facilities and programs in the newly developing areas in the east end of St. Croix. In 1974, Governor Melvin H Evans, negotiated the land transfer and a charitable trust was created. The people of the Virgin Islands and its visitors received in perpetuity a valuable piece of seashore property with its historic buildings and artifacts.

In 1974, funds were appropriated for the VI Territorial Parks System. During this period plans were considered for the development of the EGP site into a park. Camp Arawak was allowed to utilize the area for a training school for youth. However, after Hurricane Georges in 1998, the Camp Arawak Program relocated to Christiansted. Afterwards other groups and individuals continued to use the area for overnight camping, picnics, fishing, swimming and natural history education. The second floor of the factory building of the EGP plantation is currently used as a business office and focal point for school and public natural history interpretive tours.

Future prospects

STXEEMP is the VIs' first territorial marine park and is the cornerstone to the proposed future territorial park system and possibly to a regional network of marine protected areas necessary for the best management and conservation of many commercial species (Kojis et al. 1995). It was established at a cost of over $1 million of US federal funds, $1 million territorial government matching funds and in kind support from NGOs worth over $0.5 million.

The park's purpose of managing the valuable marine resources on the east end of St. Croix still has not been realized. It is generally accepted that these natural and cultural resources should be preserved for residents and visitors. However, these resources need to be managed by a technically competent territorial agency managed by professionally managers dedicated to managing these resources for the people of the VI and it's visitors. It is imperative that these resources be managed in a cost effective manner with less dependency on federal assistance in a fair and impartial matter that guarantees the benefits are available for present and future generations.

Acknowledgement

I would like to gratefully acknowledge Department of Planning and Natural Resources Commissioner Robert S Mathes and Coastal Zone Management Director Janice Hodge for their support and encouragement. Without their efforts the park would not be where it is today. Financial support for the park is through National Oceanic Atmospheric Administration funding to the US Virgin Islands territorial government's Coastal Zone Management Program. Dana Wusinich-Mendez deserves particular recognition for her efforts to develop and provide support for the establishment and management of the park. Discussions with BL Kojis regarding the park's history and politics are gratefully acknowledged. Discussions with John Farchette III greatly assisted in developing the historical and cultural perspective of implementing the park. The support of the Tropical Discoveries Fund is gratefully acknowledged.

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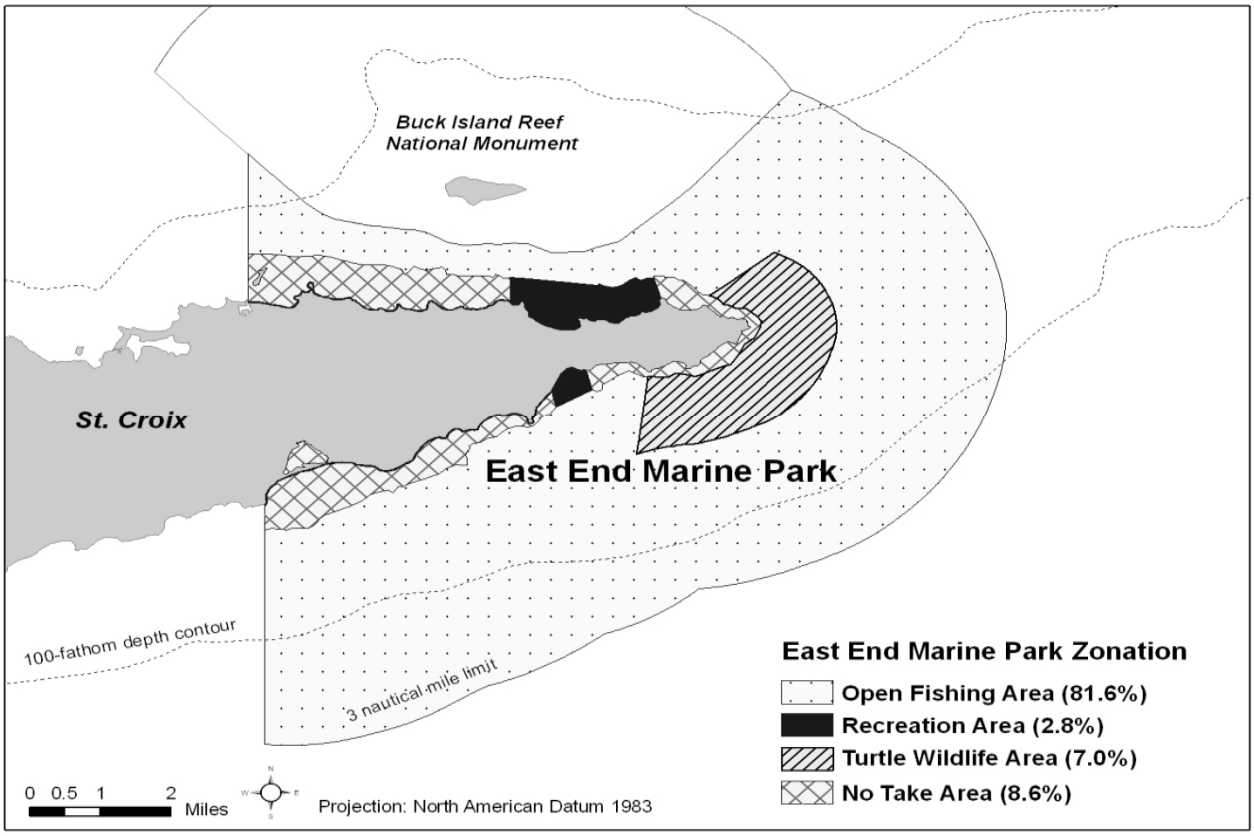

Figure 1. Multi use zonation of the St. Croix East End Marine Park (after The Nature Conservancy 2002)

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Socioeconomic and Governance Monitoring of Marine Managed Areas: A Work in Progress

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Abstract. Traditionally Marine Managed Areas (MMAs) have been established to achieve biodiversity conservation goals (e.g. protect endangered species, increase fish populations); yet increasingly social, economic and cultural objectives are being incorporated into MMA planning. To understand the effects on human communities from including socio-economic objectives in MMAs goals, a cross-site study was designed to examine the socioeconomic and governance effects of MMAs in four countries (Belize, Brazil, Fiji and Panama) as well as ecological factors influencing these outcomes. A socioeconomic and governance monitoring survey is being conducted in each of the MMA coastal communities to understand conditions prior to MMA establishment compared with current conditions. The analysis will assess the MMA effects on food security of coastal residents, livelihoods, human threats to the marine resources, and environmental awareness and knowledge as well as assess the governance effects, including impacts on management structures and strategies, stakeholder participation and representation, property rights and access to resources, management plan compliance by resource users. In addition, enabling factors for MMA effectiveness will be determined. The factors being examined include leadership and long term support of local government, participation by community and all those affected, shared benefits among participants, and alternative livelihoods that may be appropriate in decreasing resource use pressures within MMAs. This project within the Conservation International Marine Management Area Science Program, is a work in progress and demonstrates the application of monitoring and research to support improved understanding and adaptive management of coral reefs and related ecosystems.

Key words: governance, marine protected area, monitoring, socioeconomics.

Introduction

Marine managed areas[1] (MMAs) are used throughout the world to cope with human impacts on marine ecosystems by limiting unsustainable economic activities, reducing user conflicts, and promoting non-extractive uses such as recreation activities. Marine conservation efforts are recognized as working with over 75% of respondents in Caribbean and Southeast Asia acknowledging marine area conservation benefits (Loper et al. 2008). An understanding of socioeconomic and governance conditions is vital for effective implementation of MMAs.

Socioeconomic and governance monitoring is a critical tool with which coastal and ocean resource managers can determine the impact and effectiveness of their management programs. It does little good to understand the health of coral reef and other coastal resources without understanding how people are interacting with those resources or what those resources are worth to local communities. Lloret and Riera (2008) examined the evolution of human uses ini the marine environment of Cape Creus and emphasized the increasing socioeconomic importance of recreational activities in detriment to artisanal

[1] Marine managed area (MMA) and marine protected area (MPA) each have the same base definition, MPAs are primarily intended to protect or conserve marine life and habitat, and are therefore a subset of marine managed areas (MMAs), which are broader groups of named, discrete geographic areas along the coast that protect, conserve, or otherwise manage a variety of resources and uses, including living marine resources, cultural and historical resources, and recreational opportunities. Marine managed area classifications include state water quality protection area, state marine cultural preservation area, and state marine recreational management area (as defined in Fish and Game Code Section 2852. Source: http://www.dfg.ca.gov/mlpa/defs.asp)

fisheries. Apparent threshold levels of dependence on marine resources, material wealth, distance to markets and population, exist when considering customary forms of resource management (Cinner et al. 2007). On the sustainability of social-ecological systems, certain attributes of governance function in society to enhance the capacity to manage resilience in marine (Lobel et al. 2006). Given the paucity of socioeconomic and governance data available, there is an increasing demand globally from MMA managers, decision-makers, resource users, scientists and other stakeholders to understand the socioeconomic and governance conditions of MMAs.

Both government and non-government agencies, as well as other stakeholder groups, recognize that the dearth of existing data regarding the socio-economic and governance aspects of coastal and marine ecosystem management creates challenges for effective planning and implementation. This study will demonstrate the effects of MMAs on socioeconomic and governance conditions in the surrounding communities. In addition, this study will provide socioeconomic and governance information useful for determining how marine area management is contributing to community development, including poverty alleviation and equitable sharing of benefits. The synthesis analysis will contribute to marine conservation efforts at the site, regional and global levels, especially when integrated with the ecological monitoring results.

Methods

Figure 1 presents the coastal communities in the four countries where socioeconomic and governance monitoring is being conducted. These communities were selected for this study based on stakeholder consultation on access to MMAs and use patterns or dependence on marine ecosystems. These communities and corresponding MMAs are presented in Table 1.

Data collection is currently being undertaken. This involves literature review and primary data gathering. This was done as a collaborative endeavor among the participating local institutions and stakeholder groups. Surveyed respondents (n = 2736) were from a random sample (90 to 95% confidence) of MMA user groups within coastal communities.

The selection of the socioeconomic and governance variables was based on the MMAs' management objectives contained in their respective plans. The management objectives cover relevant socioeconomic and governance aspects. In addition, a series of community level multi-stakeholder consultations were undertaken to develop a consensus on identifying and selecting appropriate socioeconomic and governance indicators to measure these effects.

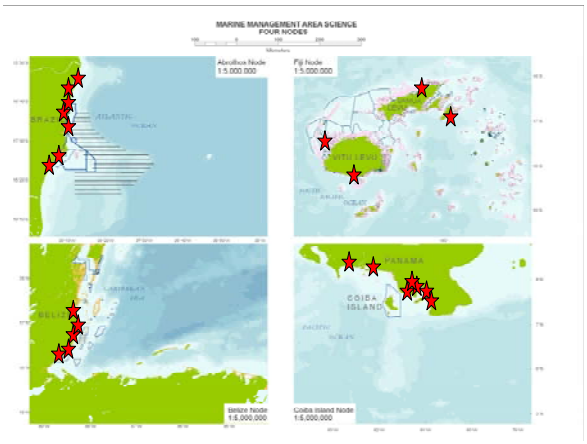

Figure 1: Location of coastal communities in the four nodes covered in this study. (counter clockwise, from top left map: (1) Abrolhos. Brazil; (2) Fiji; (3) Belize; and (4) Coiba, Panama.

Table 1: Socioeconomic effects of marine managed areas.

Country	Marine Managed Areas	Coastal communities
Belize	Lighthouse Reef, Laughing Bird Caye, South Water Marine Reserve, Sapodilla Cayes, Port Honduras Marine Reserve	Sarteneja , Chunox , Copper Bank, Dangriga, Hopkins, Sittee River, Seine Bight, Independence, Placencia, Monkey River, Punta Negra, Punta Gorda
Brazil	Albrolhos National Park, Corumbau Extractive Reserve, Cassaruba	Portu Seguro, Caraiva, Corumbau, Prado, Alcobaca, Caravelas, Nova Viscosa
Fiji	Navakavu, Waitabu, Navatu, Malolo	Waiqanake, Muaivusu, Namakala, Nabaka, Waitabu, Boumai, Lavena, Navatu, Solevu, Yaro
Panama	Coiba National Park	Pedregal, Remedios, Pixvae, Bahia Honda, Santa Catalina, Hicaco, Puerto Mutis, Leones Arriba, Gobernadora, Malena

In order to evaluate the socioeconomic impact of MMAs on individuals of specific user groups, baseline conditions prior to the establishment of MMAs were determined. Comparing the current conditions and trends against this baseline provided a basis for measuring the effect of MMAs. In the absence of existing baseline data, other benchmarks were identified.

Survey instruments were developed to measure effects on:

- Livelihood outcomes: Have the family improved economically? Have economic activities

diversified? Do households have more services available? Have the dependency on marine resource changed? Are there infrastructures changes?

- Perceptions on environmental problems: have community environmental concerns changed?
- Perceptions about and use of the MMA: are the restrictions imposed on access to resources perceived as harmful? Do communities perceive that they can take benefit from the MMA? Has knowledge about the MMA increased?
- Use of marine resources and economic activity: monthly family income, main source of income, membership in commercial organizations (coops, etc.), use of coastal resources, frequency of marine related activities, use of MMA, types of tourism activities done in the area
- Community organization and participation: are more people participating in community-based groups? Are more people participating in the MMA activities? Has the awareness of environmental/community projects increased?

In all four countries, the socioeconomic and governance monitoring sites are the same sites where ecological monitoring is being conducted. This is to ensure integration of the data for cross-disciplinary analysis of: (1) which ecological factors (e.g. productivity of extractive resources, habitat quality, ability to absorb waste, frequency of natural disturbances) affect socioeconomic outcomes (e.g. livelihoods); and (2) which socioeconomic (e.g. resource use patterns) or governance factors (e.g. stakeholder participation) affect ecological outcomes.

Preliminary Observations

Data gathering and analysis is underway in Belize, Brazil, Fiji and Panama with stakeholder consultation meetings conducted since March 2007 with local non-government organizations, MMA management bodies, and civil society groups including fishers' organizations, tour operators and tour guide associations. In addition, secondary data analysis (e.g., reports from government agencies, profiles of communities, etc.) is complete.

In Belize, fishing is not only an economic activity but a way of life, enhancing social and cultural values. More recently, the managers of protected areas have formed themselves into an association called the Association of Protected Areas Managers Organizations (APAMO). APAMO was organized to strengthen the Protected Areas Network in Belize. Through APAMO it is expected that protected areas management will evolve to focus more on a systems approach to conservation and sustainable management. Belize is a good example of co-management agreements that can be strengthened to cover key emerging issues such as stakeholder participation, conflict resolution, and recovery of investments. Creation and direct involvement of APAMO over activities in the MMA sites affect the immediate environment and ultimately, the environmental quality of the adjacent MMAs.

In Panama, the buffer zone of the Coiba National Park (CNP) has been experiencing several socio-economic and demographic changes over the past two decades. For example, fishermen in the area of Bahía Honda, Puerto Mutis, and Puerto Remedios mention having experienced a decrease in fishing productivity over the last few years which is attributed both to overfishing and to tighter regulations restricting fishing in areas of the CNP. This has implications on how some groups within communities are better positioned to take advantage of conservation benefits compared with others. There is a general positive sense about the park because it can strengthen the community (e.g.., diversifying the economy), however, there is some concern about the unequal distribution (e.g., permits to non-artisanal fishers) of benefits that may accrue only to the tourism sector.

Homogeneity of user groups across countries may have implications on the causality of effects of MMAs. Initial statistical runs for Belize and Panama show that there are significant differences in terms of four demographic parameters: household size, years of residence, age, education. In terms of perceptions of non-monetary benefits from MMAs, local communities in Belize consider marine ecosystems important, not only for extractive uses, but also for their non-use values (Figure 2).

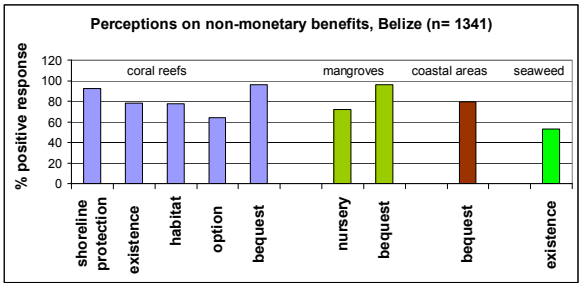

Figure 2: Perceptions on non-monetary benefits of marine managed areas in Belize.

Conclusion and Next Steps

Socio-economic and governance conditions provide the context within which coastal marine resources are managed. The values, perceptions and behaviors of local communities, key stakeholder groups and the policies developed and enforced by local and national authorities determine which areas are managed, the

extent to which they are managed and the level of compliance with management objectives. Changes in resource use and conservation patterns are therefore intricately linked to the overall political and socio-economic seascape.

Increasing poverty among coastal communities, literacy rates, historical or traditional resource use patterns, the increased cost of living, the rising cost of fuel and the annual threat of natural disasters, are just some of the variables that impact on how people interact with coastal marine ecosystems. In turn, people's interactions with coastal marine ecosystems determine the development of management strategies and the resources needed to conserve these ecosystems.

The level of conflict or compliance among user groups and with MMA management is often positively correlated with the lack of viable alternative livelihoods, resulting in communities engaging in illegal and destructive extraction activities. Hence, governance dimensions such as the type of MMA designation (e.g. ,national park, wildlife sanctuary etc.), the type of management structure (eg: government, non-government or community-based), the implementation of environmental education programs, the development of mechanisms to maintain stakeholder participation and involvement, and the effectiveness of the national legislation framework and the legal monitoring and enforcement system determine the relationship of people and marine ecosystems.

Managers of at last three of Belize's marine managed areas have begun to scientifically collect this type of data and use it in planning for more effective resource management. Because the integration of social science methodologies and approaches is groundbreaking for the conservation community, much learning regarding effective methods of study is taking place.

Data gathering in all four countries is expected to be completed in March 2009. Analysis will be completed by June 2009. The results from the synthesis of the socioeconomic and governance information from each of the countries, will provide information to: (1) establish a program of scientific monitoring of socioeconomic and governance processes for adaptive management; (2) measure the effectiveness of the MMAs in achieving its conservation goals; and (3) characterize and measure the effects of the MMA on ecological, socioeconomic and governance processes.

To ensure that the results from the socioeconomic and governance monitoring will feed into policy and decision-making, several products are being planned with relevant government and policy making bodies in each of the countries. For example, in Belize, the socio-economic and governance monitoring protocol is being developed for adoption by the Association of Protected Areas Management Organizations (APAMO).

Acknowledgement

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The value of coral quality to SCUBA divers in Barbados

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Abstract. The objective of this research is to estimate the economic value of coral reef quality related to SCUBA diving in Barbados. This value is derived using a stated preference survey of resident and tourist divers in Barbados conducted in 2007. In addition to a variety of demographic variables, divers were asked about their level of participation, expenditures related to travel and diving and encounters with specific species. Divers also identified characteristics of their most recent dive including price, crowding, fish diversity, encounters with marine turtles and coral reef quality and indicated their maximum willingness to pay for the dive. Coral quality was represented via a series of photographs representing a known range of coral cover. Hence, a quantifiable measure of coral cover is presented in a qualitative fashion that divers can understand. Results indicate that willingness to pay for increased coral quality varies with diver experience and the quality of their most recent dive. The results of this study can be used to inform management decisions regarding reef use and can aid in the development of policies aimed at maximizing the returns from diving while reducing the negative impacts of tourism activities.

Keywords: Coral quality, willingness-to-pay, marine turtles

Introduction

Barbados, like many tropical small island developing states, relies heavily on healthy coral reef ecosystems to maintain its shoreline and world famous white sand beaches, support nearshore artisanal fisheries and the tourism industry on which the GDP is now largely dependent (National Commission on Sustainable Development 2004). However, the very activities that depend on the reef, have taken their toll on reef condition, particularly those nearshore, and these reefs are now generally considered to be degraded through eutrophication, heavy use and over-harvesting (Government of Barbados 2002). This has compromised the resilience of Barbados' reefs to withstand additional external threats such as mass bleaching from elevated water temperatures associated with the global warming trend. The need for more effective conservation and management of the reef ecosystem in Barbados is clear, urgent and recognized by the Government (Coastal Zone Management Act 1998).

The purpose of this work is to understand the economic value of marine amenities in Barbados including coral reef quality, fish species diversity and marine turtles through econometric modeling of SCUBA divers' willingness to pay for dive quality characteristics. The analysis presented here represents a preliminary view of one component of a larger study examining the economic value of coastal and marine resources in the Caribbean.

Background and Literature Review

Coral reefs in the Caribbean provide a wide range of goods and services including seafood, numerous recreation opportunities, habitat, and coastal protection. Reefs also provide benefits indirectly via the jobs, income, and tax revenue generated via fisheries and marine tourism, and support human lives and livelihoods through the provision of food and materials, nutrient cycling, waste processing, and other essential goods and services. Coral reefs also have value in terms of their historic, cultural, medicinal and ecological significance.

The economic value of coral reef ecosystems is widely recognized as significant, receiving considerable attention in the popular press and academic literature in the past two decades. Numerous studies have used economic valuation methods to estimate willingness to pay for coral reef quality and the costs associated with coral reef degradation. Cesar et al. (2003) provide estimates of the value of coral reefs throughout the world, while Cesar (2000) and Gustavson and Huber (2000) provide summaries of the coral reef valuation

literature. Applications in the Caribbean include Dixon et al (1993), Spash (2000) and Parsons and Thur (2007). Brander et al. (2006) provide a meta-analysis of the recreational value of coral reefs from the literature through 2006.

Despite this attention to and recognition of value, coral reefs throughout the world continue to suffer from over-use and degradation. In the Caribbean, NOAA estimates that only 25 percent of reefs are in good health (Waddell and Clarke 2008). That the source of such significant value continues to be degraded is in large part due to the open-access nature of most reef ecosystems coupled with the failure of markets to properly allocate these resources to their highest valued use or provide proper incentives for conservation. Understanding how and why society values coral reef ecosystems can help alleviate the dearth of conservation-based management by calling attention to the degree to which reefs are undervalued and highlighting differences between potential and actual economic benefits derived from reefs. As is pointed out in Brander et al. (2006), coral reef values associated with recreation vary significantly across location and type of use, hence it is important from a policy perspective to have estimates of localized value.

Data

From June 2007 to April 2008, SCUBA divers were interviewed at dive shops in Barbados at the conclusion of their dives. In order to prevent bias associated with choice-based, on-site sampling, the distribution of surveys was matched with the exogenously determined distribution of actual divers across time and dive shop locations. In addition to a host of demographic information, divers described their most recent dive in terms of quantitative measures of fish species encountered, price paid, on-site crowding, marine turtles viewed, quality of coral cover viewed (via photo representation), and maximum willingness to pay for the dive. Divers also described their perceptions of dive quality using a series of five-point Likert scale ratings for water quality, amount of living coral on reefs, on-site crowding, level of supervision and amount and diversity of fish species encountered.

Nearly 70 percent of the 114 divers interviewed were male. The average age of divers in our sample was 38 years. Most divers in our sample were highly educated. Approximately 50 percent of the divers interviewed were visiting Barbados for the first time while only 13 percent were visiting the Caribbean for the first time. Over 57 percent of divers interviewed stated that they took vacations for the purpose of SCUBA diving. Divers in our sample have logged an average of 46 dives in the Caribbean over their lifetimes and had been diving for an average of seven years. Our sample of divers therefore has a varying degree of dive experience, Caribbean diving and general familiarity with the Caribbean.

While in Barbados, these individuals participated in numerous other recreations including beach going (79 percent), swimming (71 percent), swimming with turtles (26 percent), snorkeling from shore (41 percent), snorkeling from a boat (25 percent), sailing (19 percent), motor boating (17 percent), jet skiing (12 percent) and waterskiing (10 percent). Divers paid an average of US $104 for their 2-tank dive, had an average stated maximum willingness to pay of US $163, and expressed a high level of overall satisfaction with their dives in Barbados.

Methods

In order to estimate diver willingness to pay for coral reef quality, we regress stated maximum willingness to pay on dive quality variables, including a variable representing coral cover. This variable was acquired by showing divers four pictures of coral reefs and asking them which picture best represented the coral cover they encountered on their dive. These pictures were of reefs in Barbados where actual coral cover has been measured; actual coral cover ranged between five and 35 percent. Using this simple method however, proved ineffective as the quantitative measure of coral cover was not a significant determinant of willingness to pay. Examination of the data revealed the cause of this seemingly counterintuitive result: experienced divers viewed relatively high coral cover as poor while inexperienced divers viewed relatively low coral cover as good. That is, similar to results found elsewhere in the literature (e.g. Dearden et al. 2006), perceptions of coral quality varied greatly according to diver characteristics, including experience, number of visits to the Caribbean and participation in diving clubs and marine conservation organizations.

To account for variation in diver experience and other individual characteristics that may affect quality perceptions, we employ a two-stage econometric estimation of willingness to pay for coral cover. In the first stage, we regress divers stated satisfaction with coral cover (five point scale) as a function of actual coral cover viewed (measured coral cover in chosen photo representation) while controlling for experience and a host of individual and dive characteristics. Because the objective of this first stage of the estimation is to form a useful and accurate proxy for satisfaction with coral cover while controlling for diver characteristics, we are less interested in interpreting the coefficients from this model than in using the fitted values in our estimation of willingness to pay in the second stage. As such, we leave

statistically insignificant variables in the first stage model, noting that all variables in the model contribute to fit, and that the lack of individual coefficient significance may simply be the result of multicollinearity. In the second stage, we use the fitted values of satisfaction from the first stage as an independent variable in the estimation of maximum willingness to pay. Willingness to pay for quantitative improvements in coral quality can then be estimated by incorporating a hypothetical change in coral cover into the first stage (satisfaction) model and then adjusting the resulting fitted value in the second stage (willingness to pay) model.

Results

Results from the first stage (satisfaction with coral cover) model are shown in Table 1. When controlling for dive and diver experience variables, perceptions of coral quality are positively and significantly related to actual amount of coral cover viewed. Notably, divers visiting Barbados and the Caribbean for the first time, who were more satisfied with the dive overall, had a lower level of dive certification, and belonged to dive clubs, had better perceptions of coral quality. Males and divers who viewed Barbados diving as worse than most places where they have dived, tended to view coral quality as less favorable. Importantly for our purposes, the actual level of coral cover viewed is a positive and highly significant determinant of diver rating of coral quality while controlling for these other variables.

Table 1: Parameter Estimates Satisfaction with Coral Model

Variable	Coefficient	P-value
Intercept	1.66**	0.02
Clear sky indicator	-0.64***	0.004
Partly cloudy sky indicator	-0.25	0.15
No wind indicator	0.44*	0.1
Light wind indicator	0.15	0.32
First visit to Barbados indicator	0.51***	0.003
First visit to Caribbean indicator	0.42**	0.05
Take dive vacations indicator	-0.20	0.19
Gender indicator (male)	-0.30**	0.05
Boating this trip	0.35*	0.08
Sub ride this trip	-0.90***	0.007
Jet ski this trip	-0.19	0.45
Water ski this trip	0.60**	0.03
Played golf this trip	0.44*	0.07
Went swimming this trip	0.36**	0.03
Swam with turtles this trip	-0.61***	0.001
Tanks usually used diving	0.30*	0.07
Level of dive certification	-0.16**	0.03
Subscribe to dive magazines	0.62	0.20
Number of dive magazines	-0.548	0.18
Belong to dive clubs	0.58**	0.016
Rating of fish diversity this dive	0.40***	0.0001
Rating of viewing big fish this dive	-0.12	0.18
Rating of dive site crowding	-0.17*	0.05
Rating of satisfaction with dive	0.33***	0.005
Rating of Barbados diving (high = worse)	-0.27***	<.0001
Charter price includes gear rental	0.26	0.11
Coral cover viewed during dive	0.02***	0.002
R^2 = 0.773		

*** significant at 1% level, ** significant at 5% level, * significant at 10% level

The coefficients shown in Table 1 were used to generate fitted values of coral quality rating for each diver in the sample. These fitted values were then used as an independent variable along with the number of marine turtles viewed, the level of on-site crowding (number of other divers at the dive site), number of fish species encountered, and income, in a model of willingness to pay. These results are shown in Table 2. Divers' maximum willingness to pay is positively and significantly related to their perceptions of coral quality (modeled in stage 1), and the number of turtles encountered. On-site crowding significantly decreases willingness to pay. The relatively low goodness-of-fit in the second stage estimation is not unexpected given the small sample size and small number of explanatory variables included in the model, and is consistent with other estimates of willingness to pay in the literature (Asafu-Adjayea and Tapsuwan, 2008, for example).

Table 2: Parameter Estimates Willingness to Pay Model

Variable	Coefficient	P-value
Intercept	97.22*	0.08
Predicted value of coral quality rating (1st stage)	30.60***	0.01
Number of turtles encountered	10.35*	0.10
Number of fish species encountered	-1.32	0.39
Crowding (number of other divers on site)	-5.17**	0.05
Income	-0.0003	0.20
R^2 = 0. 20		

*** significant at 1% level, ** significant at 5% level, * significant at 10% level

Conclusion

In order to use these results to approximate willingness to pay for improvements in coral cover, we work through both stages of the modeling effort. For example, a 10-percent improvement in coral cover would result in a 0.2 increase in the predicted value of coral quality rating. When incorporated into the willingness to pay model, this results in willingness to pay increasing by approximately US $6.12. Hence, our results suggest that divers are willing to pay an additional US $6.12 per two-tank dive for a 10-percent improvement in coral cover in Barbados. Other notable results include divers' willing to pay US$10.35 per dive per additional marine turtle viewed, and US $5.17 per dive to avoid sharing dive boat and dive site with an additional diver.

Between 30,000 and 50,000 divers visit Barbados each year. If each diver participates in only one dive per visit to Barbados, additional economic value from dive tourism from a 10 percent increase in coral cover at dive sites could be as high as US$306,000. This estimate is most likely a lower bound on the true value to divers of such an improvement, as this estimate assumes the number of divers remains constant following an improvement in coral quality

and assumes each diver dives only once per visit. Coupling this result with divers' willingness to pay to avoid crowded sites would suggest that managing and restricting use at dive sites would both improve coral quality and economic gains.

Coral reefs in Barbados and throughout the Caribbean provide a significant source of economic value. Yet, coral reefs are declining in quality and may not be given due attention in policy formation and land use decisions. This work has shown that significant economic gains can be realized through efforts to improve coral quality.

Acknowledgements

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Managing Indonesian Coral Reefs: Lessons from Coral Reef Rehabilitation and Management Program

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Abstract. In 1998, the Indonesian government launched the Coral Reef Rehabilitation and Management Program (COREMAP) with the objective to enhance the health of coral reefs and to improve the welfare of coastal communities depending on coral reefs. The first National Policy, strategy and action plan was established in 2001 and implemented in 15 districts of 7 provinces. Scientific monitoring of coral reefs and socio-economic condition of dependent surrounding communities were carried out. Results indicated an improving trend in terms of live coral cover. Scientific results will be translated into popular articles in simple language which can be easily understood by all levels of stakeholders to enhance understanding of the importance of coral reefs. Components of community awareness and community control produced the most significant impact by reducing rates of degradation. Handing over management rights and control to local community stakeholders have proven efficient by increasing fisheries yield and community income. Television media and the involvement of community leaders proved the most efficient awareness media. Involvement of government officials in the awareness campaign activities has increased their appreciation of the importance of coral reefs for the people.

Key words : Coral Reef , Lessons Learned, Management

Introduction

Indonesia is the largest archipelagic country in the world (5000 km long, 2000 km wide) composed of 18,110 islands with a coastline of 108,920 km. Two shallow seas (the Sunda shelf in the west and the Sahul shelf in the east) are separated by a deep ocean that has existed since the Eocene (Tomascik et al, 1997). The Sulawesi, Flores and Banda Seas never dried up, in contrast to the Sunda and Sahul shelves, providing a chance for the marine biota to evolve continuously. Thus the waters surrounding the Sulawesi and Banda Seas are now one of the centers of biodiversity and Indonesia contains about 14 % of the world's reefs which are distributed unevenly from Sabang to Merauke with the highest concentration around Sulawesi and Banda seas.

More than 5 million Indonesian citizens depend on shallow water fisheries. Most of them are traditional fishermen who live at a subsistence level and depend fully on coral reefs resources. Despite this significant dependency, coral reefs in Indonesia are under serious threat from destructive fishing, bleaching events and pollution.

Aware of the importance of coral reefs to Indonesian society and the livelihoods of millions of coastal dwellers , and that reefs in Indonesia are under serious threat, the Indonesian government introduced a policy, strategy and action plan to improve the management of coral reef ecosystems. This paper tries to share the experience and the learning process to manage coral reefs in a developing country like Indonesia, starting from drafting the policy, strategy and action plan and its application process.

National Policy, Strategy and Action Plan

Drafting of the plan involved stakeholders at national level (academics, government officials, NGOs).as well as from all over Indonesia. After due consideration of inputs at national and local levels, a National Policy, Strategy and Action Plan was finally agreed upon, with the main objective of managing the coral reef ecosystems based on the balance between conservation and utilization. This plan was to be designed and implemented in an integrated and synergistic manner by the central and regional governments, civil societies, private sectors, higher education institutions and non government organizations. This National Policy, Strategy and Action Plan (NSPA) comprised 7 policies, 9 strategies and 34 action plans. Better known as "Coral Reef Rehabilitation and Management Program", it is sub-divided into three execution phases (initiation phase of 3 years, acceleration phase of 6 years and institutionalization phase of 6 years; COREMAP

LIPI 2001). The Action Plan of this program has five components: community awareness and education, control and surveillance, research and monitoring, community-based management, and institutional strengthening.

The COREMAP program was implemented in 7 Provinces (15 Districts) since 1998. The NPSA was made acceptable to all concerned and a legal basis for coral reef management was established. After much advocacy ranging from local communities to members of parliaments and the President, Constitution No. 27 of 2007 was decreed by the State Minister of Marine Affairs and Fisheries.

Research and monitoring included an education and training program as well as the establishment of an Information and Training Center (CRITC) at which 727 trainees (512 men and 215 women) were trained. Monitoring results of reefs appear to show no significant declines in reef quality (Table 1, Figs. 1 and 2). Coral reefs distribution was mapped using Landsat TM, resulting in imagery of ~19,500 km^2. 590 coral species in 82 genera or about 80 % of the world's total coral fauna (Fig. 3) were found.

Table 1. Status of Coral Reef Condition based on Percentage live Coral Cover from 77 areas and 908 stations (over what time frame?) [change commas to decimal points in % cover)

Location	Stations	Excellent	Good	Fair	Poor
West	362	5.5	27.1	33.9	33.4
Central	274	5.1	30.3	44.9	19.7
East	272	5.9	17.3	34.2	42.6
Total	908	5.5	25.1	37.3	32.1

Utilization pattern of coral reefs in Indonesia may be differentiated into three categories, (i) fishers who utilize coral reefs at one place for a certain time before moving on to other sites; (ii) fishing groups who use coral reefs at a fixed locality only; and (iii) those who fish periodically far away from their home. Relocation was motivated by the destruction and decrease in productivity of coral reef resources. This migrant fishing includes moving the entire family from one site to the next over a period of years. The migration cycle starting from the search for new locality rich in fish resources, followed by moving the whole family and developing a new settlement until the depletion of the resources and starting over again extends over some 50–60 years. Annual average population growth rate in coastal villages is 2.24% and migrant ratio 10-50%. (Kramer and Simanjuntak, 2000). Roaming fishermen move among islands during trips taking 3 – 6 months. This group has the highest potential for reef destruction due to absence of sense of ownership and emotional attachment to the location of exploitation and consists often of Bajo, Bugis and Madura clans who travel the entire Indonesian waters (Figure 4). Improved community awareness and provision of alternate incomes were identified as key prerequisites to alter destructive behavior and the program had some successes.

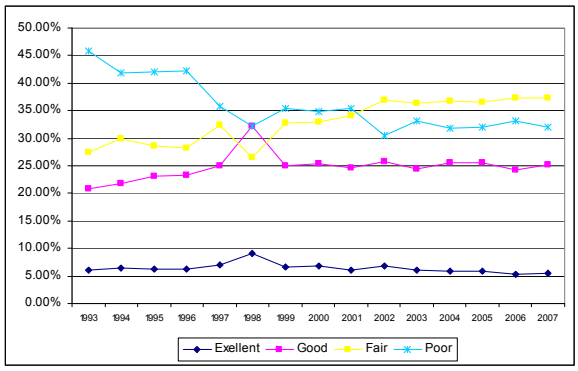

Figure 1. Trend of Coral Reef Condition (1993 – 2007)

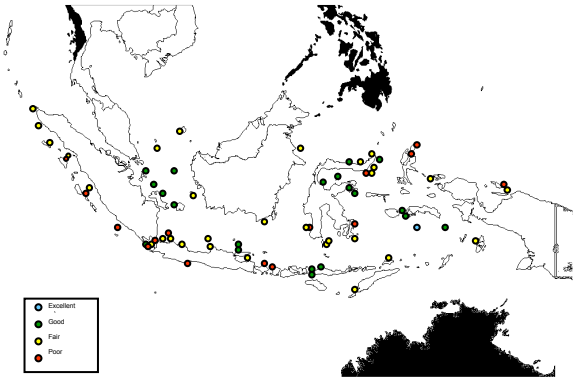

Figure 2. Coral Reef Condition in Indonesia [legend impossible to read—please fix figure]

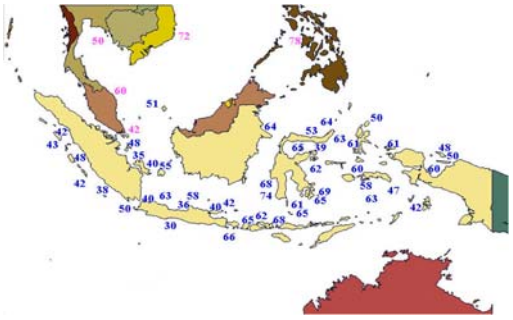

Figure 3. The Distribution of Coral genera in Indonesia.

Rivalry between the local institutions and minimal knowledge of officials and communities regarding conservation-based management principles proved challenging. Existing institutions were targeted for education and capacity building and the program concentrated on enhancing knowledge and skill in prevention of reef degradation as well as sustainable use of the

resource. Increased community participation led within three years to increased catch which in turn enhances community income after management rights were conferred to the coastal community in Mapor, Riau Province (Mujiyani, 2007)

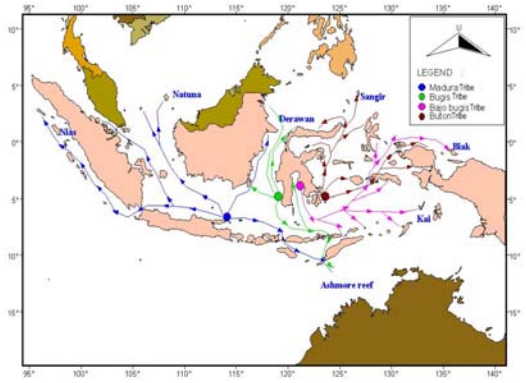

Figure 4. Route of Sea Wondering Fisherman of Madura, Bugis, Buton & Bajo Tribe

All fishermen that joined the COREMAP program understood and voluntarily follow the program, fully realizing the importance of managing coral reef resources. Conflict occurred with non residence fishermen taking resources managed by the local fishermen. Thus acknowledgment of the local community's management right and full transfer of responsibility are important. Control by local community proved efficient from psychological and budgetary viewpoints, since the community was able to control extensive areas at a reasonable cost. Lack of coordination between community officials and law-enforcement officers was observed and negated many fledgling successes.

Control and community awareness programs reduced fishing activities using explosive. While in the past, bombers were considered friendly by the local populace (bombers took only big fish allowing the population to collect the small ones), this attitude is beginning to change The immediate result is that the coral reefs located near human settlement seem to be is in better condition than the more remote ones. This condition is a reversal of that observed a decade ago where the coral reefs near human settlement were the most severely degraded.

Also a community awareness program was directed to all level existing in the communities starting from the decision makers, member of parliament, President, to the local community inhabiting the coastal areas. Overall 63% of the general public and 71% of the coastal communities were aware of the "Save our Coral Reef" campaign (Coremap, 2002). Most effective media were TV and radio programs. In coastal communities and local governments campaign events or a visit by a prominent figure proved efficient.

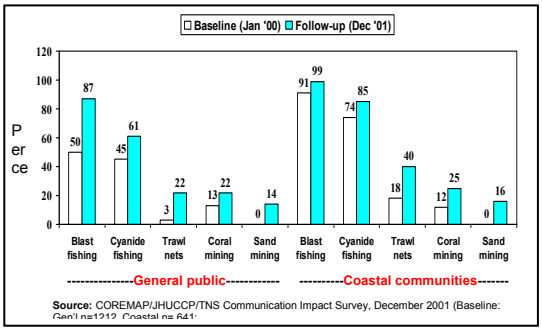

Figure 5. Percent who regard specific fishing techniques as threatening to coral reefs, 2000-2001

Lack of education and scientific knowledge of the coastal communities was a main causes for marine resources degradation.. In Indonesia, formal education curricula are characteristically land-oriented and similar for all schools, whether located on the coastal or far inland. Aware of the importance of formal education, text books on marine life were distributed. For the 6-year elementary school, 7 books; one a teacher-guide, six for grades 1 to 6, were produced. The Central Education Service was involved and the books have been adopted by the Local Education Services.

While coral reef conservation in Indonesia remains challenging, our program has shown that society-wide information, increased education and empowerment of local resource stewards has helped to significantly advance conservation goals.

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Post-hurricane assessment of reefs and banks in the northwestern Gulf of Mexico

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Abstract. This study characterized and compared the benthic habitats of four banks in the northwestern Gulf of Mexico (Sonnier, McGrail, Geyer, and Bright Banks) and documented potential hurricane damage at these banks. Hurricane Rita, a Category 3 storm, passed 12 km east of McGrail Bank and 24 km west of Sonnier Bank on September 23, 2005. Hydrodynamic hindcast models indicated water velocities of 4 m/s in the vicinity of the banks and estimated maximum wave heights of 13–26 m, which could have exposed even the deeper banks. Results of this study revealed that Sonnier Bank exhibited the lowest live cover at all depth ranges (2–38%) and the highest diversity of all banks, while Bright Bank exhibited the highest live cover (86%). Live cover was significantly different across banks and multivariate statistical analyses using benthic cover data showed significant differences between banks. No obvious signs of hurricane damage were observed at McGrail, Geyer, and Bright Banks; however, a loss of live cover was observed at Sonnier Bank. The results of this study suggest that hurricane impacts to algal and sponge dominated banks may be harder to detect more than a year after a hurricane has passed.

Key words: hurricane, McGrail Bank, Geyer Bank, Sonnier Bank, Bright Bank, Gulf of Mexico

Introduction

Hurricane Rita passed through the Gulf of Mexico in September 2005. Several sensitive habitats within the northwestern Gulf of Mexico were in close proximity to the path of Hurricane Rita, including Sonnier Bank (SB), McGrail Bank (MB), Geyer Bank (GB), and Bright Bank (BB), which were all located within ~75 km of the storm track (Fig. 1). Suspected large wave heights may have exposed some bank caps, even those located at depths of 20–30 m. Preliminary assessments of the coral reef cap (18–29 m) at the neighboring East Flower Garden Bank (EFGB) following Hurricane Rita indicated substantial mechanical impacts: fractured and displaced corals, sediment-scoured corals bordering sand flats, and corals scarred and dented by waterborne objects (Hickerson, EL and Schmahl, GP, pers. comm.). The goals of this study were: 1) to characterize and compare the benthic habitats within four depth ranges at SB, MB, GB, and BB; and 2) to document potential hurricane damage at these banks. Additionally, it was an important goal of this study to distinguish between storm damage and damage from oil and gas activities, which might be apparent at greater depths.

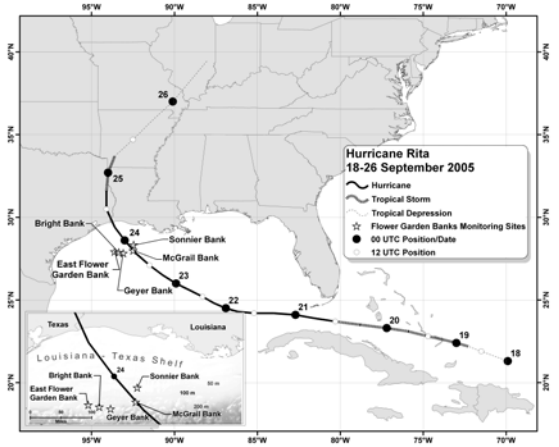

Figure 1: Track of Hurricane Rita, 18–26 September 2005 in relation to the banks evaluated in this study. Image courtesy of A. Hazra.

Material and Methods

In order to understand the wave and current conditions in the vicinity of SB, MB, GB, and BB during the passage of Hurricane Rita, a wave study was conducted using hindcast data provided by Oceanweather, Inc. This study consisted of two distinct analyses: (1) a numerical modeling effort using the REF/DIF program to determine the magnitude of wave transformation from the hindcast wave location to the bank locations and (2) an analytical model to estimate the potential currents resulting from the transformed wave conditions.

In April and May 2007, videographic transects were collected by divers and a remotely operated vehicle (ROV) to characterize the benthic habitats of SB, MB, GB, and BB. Previous descriptions of biological communities at banks in the northwestern Gulf of Mexico (Rezak et al. 1985, Boland 1999, Rezak and Bright 1979, Schmahl and Hickerson 2006) were considered during the sampling design for this study.

Four distinct depth zones were sampled within the habitat types described by Rezak et al. (1985; Table 1). The depth discontinuities between the four sampling zones were purposely established to prevent overlap at the margins of each depth zone.

Table 1. Summary of data collected by divers and ROV in four depth zones. D = sampled by diver; R = sampled by ROV; NS = not sampled; "-" = zone not present; number following dash represents number of transects.

Depth Zone (m)	Bank			
	Sonnier	McGrail	Geyer	Bright
22–27	D–2	-	-	-
30–36.5	D–8	-	D–8	D–3
45–50	R–7	R–12	R–4	NS
55–60	R–6	R–8	R–7	NS

Divers videotaped transects within no-decompression diving limits (to ~36.5 m depth). Video footage was collected at a height of 40 cm and perpendicular to the substratum using a digital video camera in an Ikelite® underwater housing fitted with a wide-angle lens and underwater video lights (Aronson et al. 2005). A TrackLink® 1500 High Accuracy Ultra Short Baseline acoustic positioning system (USBL) with two beacons and an accuracy of 0.25 degrees was used to track the location of videotaping divers/ROV. Data were converted into a GIS format to visualize the location of diver/ROV transects in geographic space (Fig. 2).

Eight randomly positioned transects (each 10 m long) were videotaped at SB within the 22 to 27 m depth range. Because these transects were positioned with reference to a single line (pseudoreplicates), the data from these eight transects were pooled, creating a single long transect for statistical analysis. An additional transect was laid in this depth zone, beginning 21 m northeast of the U-bolt and videotaped in a 90° direction. In the 30–36.5 m depth range at SB, GB, and BB, transects were set along depth contours. Continuous and unidirectional video footage began at random locations.

ROV video footage was collected along depth contours in the 45–50 m and 55–60 m depth zones at SB, MB, and GB using a Seabotix LBV 300S-6[2] ROV. The TrackLink USBL system was used to track the ROV and the position of the ROV was recorded in real time. Due to impending foul weather, ROV data was not collected at BB.

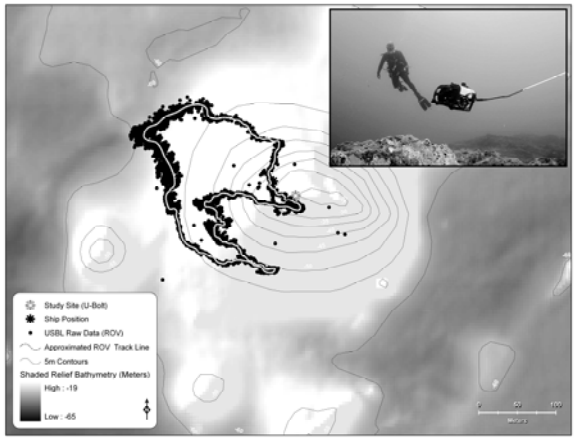

Figure 2: Map of ROV track at Sonnier Bank to and from the ship to a maximum depth of ~60 m. Image courtesy of A. Hazra.

To capture landscape-scale views of all four banks, divers recorded video footage during their descents and along the seafloor. Roving diver video was recorded at 1–2 m above the substrate, while looking down at a 45° angle. The roving diver video was qualitatively analyzed for possible hurricane impacts and compared to video from previous surveys, which acted as an estimate of pre-hurricane conditions and benthic community structure. The ROV also collected landscape-scale views at deeper depth ranges.

Transect video footage collected at SB in the 22–27 m depth range was cut into non-overlapping still images using ULead® VideoStudio® 9. Each 10 m transect yielded ~4 m^2 of benthic imagery. For contour video collected at all other depth ranges, random start times were used to generate 37 consecutive still images from the video footage (4 m^2), which were equivalent to one 10 m transect. A random number of frames were skipped before beginning the next 37 frame transect. Randomly placed dots were then added to each frame using Coral Point Count® (CPCe), for a total of 500 dots per transect. Organisms positioned beneath each random dot were identified to the lowest possible taxonomic level. Quality assurance/control consisted of

independent analyses by trained observers on the same segment of captured video. There was agreement between pairs of observers at least 95% of the time.

Results

Hydrodynamic Model Predictions

The REF/DIF model was run to determine the potential effects of banks on waves generated by the storm. Results showed that wave heights increased dramatically in the vicinity of the banks due to the large difference in water depth between the seabed and the summit of the bank. The modeling effort suggests that wave heights are potentially increased to just below the wave-breaking point in the vicinity of the banks. Therefore, the wave heights from the hindcast study were increased to just below breaking—approximately 60% of the water depth at each bank location—in order to estimate the water velocities induced by the waves on the banks. These transformed waves were then input into the Stream Function Wave Theory program to estimate water velocity. Table 2 displays the maximum bottom velocity and maximum wave height at each bank.

Table 2. Results of the hydrodynamic modeling effort in relation to bank depth and distance from the Hurricane Rita storm track.

Bank	Bank Depth (m)	Distance from Storm (km)	Max Bottom Velocity (m/s)	Max Wave Height (m)
McGrail	45	12	4.0	26
Sonnier	22	24	4.0	13
Geyer	39	58	4.0	26
Bright	33	75	4.5	20

Benthic Characteristics

Live cover data were pooled for each bank and data were square-root transformed in order to perform a one-way analysis of variance (ANOVA). Live cover was significantly different across banks ($F = 16.49$, $df = 3,61$, $P<0.0001$). Tukey's HSD test showed significant differences between all pairs containing SB, as well as between BB and MB (Table 3).

Table 3. Tukey's HSD test performed on square-root transformed live cover estimates from diver and ROV transects. Asterisk (*) denotes significant difference at $\alpha = 0.05$; ns = not significant.

Bank	Sonnier	McGrail	GB	Bright
Sonnier	–	*	*	*
McGrail		–	ns	*
GB			–	ns
Bright				–

Sonnier Bank, the only bank in this study located east of the storm track, exhibited the lowest live cover at all depth ranges (~2–38%) when compared to MB, GB, and BB (~17–86%). SB was dominated by brown macroalgae, sponges, and *Millepora alcicornis* in shallow areas, while deeper zones were dominated by "fine turfs (< 3 mm) and bare space" (TB) and "rubble" (rock or coral rubble - bare or covered in fine turf algae). With increasing depth, percent live cover decreased at SB (22–27 m, 38.3%; 30–36.5 m, 30.1%; 45–50 m, 6.8%; 55–60 m, 1.8%). Qualitative analyses of video footage collected at SB by divers in 1996, 2002, and 2005 showed differences in benthic cover compared to video collected in 2007. Algal and sponge cover appeared to be higher in the previous years. Another notable difference was the apparent disappearance of *Xestospongia muta* colonies. This species was present in 1996, declined to one individual with disease-like characteristics in 2002, and then was not recorded in 2005 or 2007.

McGrail Bank lies only ~12 km west of the storm track and has the deepest reef cap of any bank evaluated in this study (45 m). Live cover at MB ranged from 17–38%. In the 45–50 m depth range, rubble and sand were the dominant features, while live cover (38%) was dominated by macroalgae and hard corals. The coral cover consisted mostly of *Stephanocoenia intersepta* (4.8%) with *Millepora alcicornis*, *Agaricia* sp., and *Montastraea cavernosa* comprising the remaining 0.7%. Because the percent cover of *S. intersepta* appeared to be much higher in some areas within this depth range (ranging from 0–32% within the transect data), multidimensional scaling (MDS) analysis was performed on the 45–50 m transect data based on multivariate cover of the four hard-coral taxa. The MDS plot (Fig. 3) reveals that there is indeed high variability among transects in the composition of the coral assemblage at MB in the 45–50 m depth range.

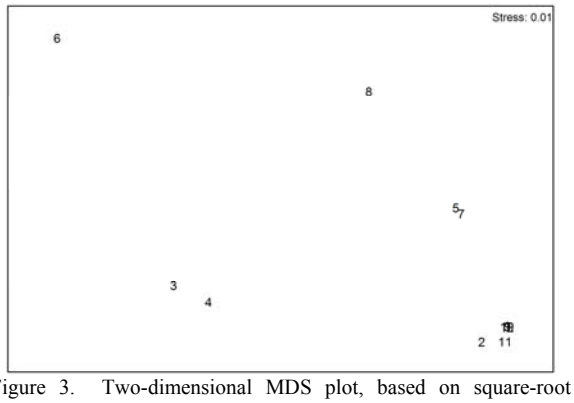

Figure 3. Two-dimensional MDS plot, based on square-root transformed Bray-Curtis similarities, comparing multivariate cover of the four hard-coral taxa at McGrail Bank within the 45–50 m depth range.

In the 55–60 m depth range at MB, rubble and sand were the major benthic components, while live cover (17%) was comprised mostly of algal nodules (6.5%) and the brown macroalga, *Lobophora variegata* (3%).

Previous videos were not available for qualitative analysis. No apparent hurricane damage in the form of overturned or injured corals was observed.

Live cover at GB ranged from 30–60% and was mostly comprised of brown macroalgae, corals, and sponges. Geyer Bank is the only bank evaluated in this study with an observed population of *Tubastraea coccinea*, an exotic, invasive, azooxanthellate scleractinian coral that has recently invaded the Gulf of Mexico (Fenner and Banks 2004). Live cover was 60% within the 30–36.5 m depth range and was dominated by brown macroalgae (42%), with *Sargassum* being the dominant genus at 27%. Rubble and TB were important contributors to overall cover. In the 45–50 m depth range, live cover was ~30%, with brown macroalgae as the dominant taxonomic group at ~22%, and rubble and TB comprising 32% and 31%, respectively, in this depth range. In the 55–60 m depth range, live cover was ~35% and was comprised mostly of brown macroalgae (22%). Rubble and TB accounted for 43% and 12% of the total cover, respectively. Diver video collected at GB in 2003 showed similar benthic cover to the video that was recorded in 2007 with no obvious signs of hurricane damage.

Due to foul weather, only one depth range (30–36.5 m) was surveyed at BB, which exhibited the highest live cover of all four banks examined in this study (86%), with brown macroalgae (44%), green macroalgae (13%), and turf algae (12%) as the dominant groups. Coral cover was ~8% and was comprised of four species: *Millepora alcicornis, Diploria strigosa, Stephanocoenia intersepta,* and *Montastraea cavernosa*. Diver video taken in September 2003 revealed mostly bare substrate, low macroalgal cover, and few large coral colonies. No hurricane damage was observed at BB.

Statistical Analyses

The Shannon-Wiener Diversity Index (*H′*) was calculated for each bank at each depth using the lowest taxonomic group possible. Diversity was highest at SB from 22–27 m, due largely to the variety of sponges present there. Diversity decreased with depth at SB (Table 4). Both GB and MB exhibited the highest diversity values in the 45–50 m depth range. The brown macroalgae accounted for these high diversity values. Notably, diversity at 45–50 m was very low at SB compared to GB and MB. Brown algae distinguishable to genus or species were virtually absent from SB between 45 and 50 m. Turf algae, rubble, and sand were the dominant categories at SB from 55–60 m, accounting for the zero-value of *H′* in that depth range. It should be noted that in this study, turf algal species were lumped into a single taxonomic unit because individual taxa were not distinguishable from video; however, the actual species diversity of turf algae would be expected to be high, and if known, would likely increase the *H′* for the deeper transects at SB.

Table 4. Shannon-Wiener Diversity (*H′*) at all banks and depths surveyed.

Depth (m)	Sonnier	Geyer	McGrail	Bright
22-27	2.86	----	----	----
30-36.5	1.55	1.65	----	1.81
45-50	0.23	2.13	2.08	----
55-60	0*	1.47	0.83	----

*only turf algae, rubble, and sand

Multivariate statistical analyses were performed using benthic cover data at SB, MB, GB, and BB. Analysis of Similarity (ANOSIM) showed significant differences between banks (Global R = 0.54, P = 0.001). Within site comparisons showed less dissimilarity between depths. MDS highlighted the dissimilarities among banks, with depths within sites grouping more closely (Figure 4).

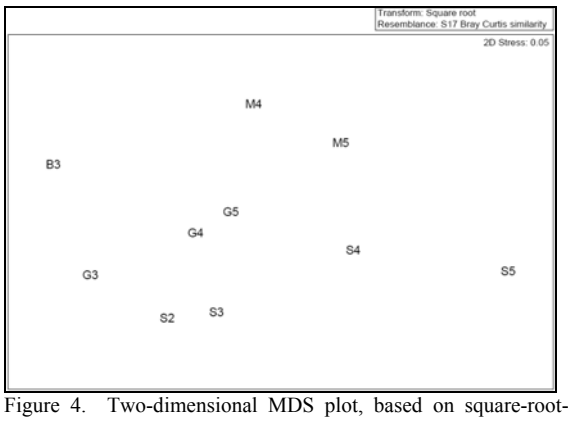

Figure 4. Two-dimensional MDS plot, based on square-root-transformed Bray–Curtis similarities, comparing multivariate benthic cover data among Sonnier, McGrail, Geyer, and Bright Banks at the depth ranges sampled. Data were pooled within depths at each site. Abbreviations: S2, Sonnier Bank 22–27 m; S3, Sonnier Bank 30–36.5 m; S4, Sonnier Bank 45–50 m; S5, Sonnier Bank 55–60 m; M4, McGrail Bank 45–50 m; M5, McGrail Bank 55–60 m; G3, Geyer Bank 30–36.5 m; G4, Geyer Bank 45–50 m; G5, Geyer Bank 55–60 m; B3, Bright Bank 30–36.5 m.

Discussion

Little is known about the effects of hurricanes on benthic communities not dominated by corals (e.g., SB, MB, GB, and BB). This study suggests that hurricane impacts to algal and sponge dominated banks may be harder to detect more than a year after the hurricane has passed. Since the passage of Hurricane Rita, algal communities have had time to re-grow and sponges may have healed/regenerated. Furthermore, corals, sponges, and other organisms that may have been removed from these banks during the hurricane were likely fractured and dispersed by the predicted water velocity of ~4 m/s, leaving little

or no detectable remnants 20 months after the storm. Vast areas of rubble, documented at SB and at deeper depths on MB and GB may have represented hurricane effects; however, this may not be the case as normal reef processes also create rubble zones on banks in the northwestern Gulf of Mexico (Rezak et al. 1985).

A wave study was conducted to better understand the wave and current conditions in the vicinity of SB, MB, GB, and BB during the passage of Hurricane Rita. The REF/DIF model wave height estimates represent the largest theoretical wave heights that may have occurred at each bank cap. Based on these results, larger wave heights may have occurred at banks with caps located in deeper water (i.e., MB and GB). However, it is interesting to note that the analysis of transect video in this study did not show the greatest damages at MB and GB, as might be expected by the results of the wave model. Furthermore, the shallowest bank included in this study (SB at 22 m depth) experienced the most observable damage, with the appearance of bare substrate and rubble fields in the 2007 transect video. While one might assume that the greatest damage would likely occur at banks located closest to the storm track, the results of this study do not support this assumption. MB is located closest to the storm track (12 km); however, no hurricane damages were observed at this bank. Significant damages were detected at SB, which is the only bank in this study located east of the storm track. It is common knowledge that the right side of a hurricane (relative to its direction of travel) is the most powerful portion of the storm in terms of wind speed and storm surge. This may be a contributing reason for the damages observed at SB.

Potential Hurricane Effects

As the shallowest bank located on the east side of the hurricane track, with hindcast water velocities up to 4 m/s and estimated waves up to 13 m, SB likely sustained hurricane damage in late September 2005. Benthic survey results showed that SB had lower live cover than any of the other banks in this study. At the shallowest depth (22 m), divers with previous experience noted that the bank surface appeared very different from previous visits. Areas of bare bedrock appeared in places previously occupied by live cover.

The depth of MB (45 m) may have protected the scleractinians and algal nodules from hurricane damage, even though the hurricane essentially passed over the bank. Wave heights of up to 26 m were modeled to have passed over MB. Undoubtedly, the pressure and velocity of water during the storm affected the top of the bank, even at 45 m depth. However, the absence of baseline data with which to compare the observations and data gathered at MB during this study prevents statistical comparisons and limits our ability to draw inferences from these data. Because ROV footage was taken at night, landscape-scale views were not possible to discern. It may be that hurricane damage was not detected for this reason. The colonies that were observed appeared undamaged; however, the high variability of coral cover observed in the 45–50 m depth range could have been a consequence of the hurricane. On the other hand, that high variability could reflect the patchiness of the benthic biota caused by variability of coral recruitment or post-settlement processes, or previous perturbations.

Video footage from 2003 was analyzed qualitatively for pre-hurricane conditions at GB and BB. The subject of both videos was the fish populations at these banks; therefore, a thorough characterization of the benthic community structure was not possible. Video from BB showed largely bare, uncolonized areas, with *Lobophora variegata* being the only discernible macroalgal genus. Video from GB showed coverage of the benthos by brown macroalgae, including *Sargassum* spp., which was the dominant macroalgae in 2007. From these pre-hurricane videos we can conclude: (1) that the unique biological characteristics were not established after the hurricane; and (2) that as of April–May of 2007, the benthic assemblages had recovered to some extent.

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Patterns of spatial variability in distribution of benthic invertebrates and algae at Sharm el Sheikh (South Sinai, Egypt)

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Abstract. A survey of benthic community was conducted as part of a project to construct an Underwater Observatory on the coast of Sharm el Sheikh (Egypt). To provide the first step of the EIA based on a "Beyond BACI" design, the spatial distribution of benthic invertebrates and algae was studied at a hierarchy of spatial scales: 5 sites, 3 areas within each site and 20 1m^2 frames within each area were randomly selected. Plots were sampled using the photo-quadrats method. A combination of univariate and multivariate analysis of the percentage cover was used to describe differences in the abundance of organisms identified at the genus level and, when necessary, grouped into broader categories. Results showed significant differences in the distribution and abundances of organisms among sites as well as among areas, indicating that abundances varied at the scales of hundreds and tens of meters along the coast investigated. These results are likely to be due to spatial differences of the hydrodynamic conditions of the studied sites, other than to stochastic events. One of the sites with the lowest percentage cover of living organisms was suggested as the most suitable to place the Underwater Observatory in order to minimize the impact of the structure.

Key words: Spatial variability, Coral reef, Red Sea, Photo-quadrats, EIA

Introduction

This study is a part of the "Pilot project for the sustainable development of environmental sound management in South Sinai, Sharm el Sheikh, Ras Mohammed National Park" funded by the Italian Ministry of the Environment. Within this project, the construction of an Underwater Observatory close to the Visitor Center of the park was proposed. In response, the coast of Sharm el Sheikh was surveyed to provide information about spatial variability in distribution of benthic invertebrates and macroalgae.

Variability in the distribution of natural species can occur at different spatial scales (Kennelly and Underwood 1992; Underwood and Petrait 1993; Underwood and Chapman 1996; Connell et al. 1997, Benedetti-Cecchi et al., 1998; Menconi et al. 1999; Ferdeghini et al. 2000; Balata et al. 2006). Documenting the spatial scales at which variability in the abundance of organisms occurs can help to focus attention on the relative importance of different ecological processes that can determine the patterns observed (Underwood and Chapman 1996).

In this study, the sampling procedure was designed in order to be the first step of an Environmental Impact Assessment based on a "Beyond BACI" (Before-After, Control-Impact; Underwood 1991) design.

The logic of this design is to separate the spatio-temporal natural fluctuations of the structure of benthic assemblages from those due to anthropogenic activities. Thus, an appropriate analysis would consist of properly replicated sampling several times before the development and several times after in potentially impacted and in reference locations. From these data, it is possible to ascertain interactions in impacted and control sites through time (Underwood 1991, 1992, 1993, 1994).

Moreover, documenting the spatial distribution of living organisms was essential to suggest the most suitable stretch of coast to place the Underwater Observatory.

Materials and Methods

The study was carried out at Ras Mohammed National Park along the coast of Marsa Ghoslani bay (Fig. 1). Blocks of organic framework of dead and living corals characterized the substrate of the study area.

A nested sampling design was used at a hierarchy of spatial scales: 5 sites (100s of m apart), 3 areas within each site (10s of m apart) and 20 replicates of 1m^2 frames within each area were randomly selected in the Northern part of Marsa Ghoslani bay (Fig. 2).

Plots were sampled using the photo-quadrats method (English et al. 1997; Acunto et al. 2001; Hill

and Wilkinson. 2004). All samples were collected at a depth of 10 m on a gently sloping rocky bottom (with an angle ranging from 10 degrees to a maximum of about 15 degrees).

Figure 1: Study location along the coast of Sharm el Sheikh, Egypt.

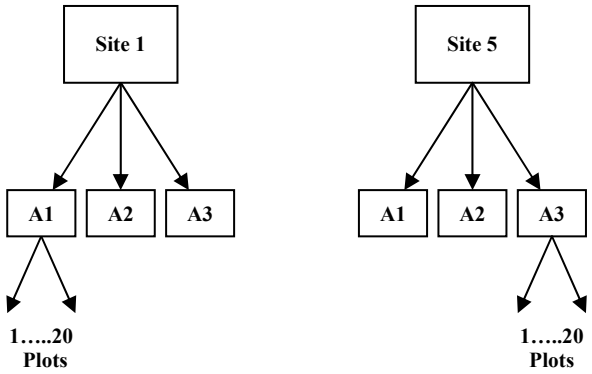

Figure 2: Sampling design.

A permutational multivariate analysis of variance was used to study the spatial variability among sites and areas by PERMANOVA (Anderson 2001; McArdle and Anderson 2001). This computer program uses permutation procedures to obtain the null hypothesis distribution (indicated as "pseudo" *F*) and *P*-values for the tests (including interactions), for any balanced multi-factorial ANOVA design. It is a non-parametric test.

The analysis consisted in a 2-way model with SITE (5 levels) as a random factor, AREA (3 levels) as a random factor nested in SITE. This analysis was performed using cover of sessile invertebrates identified at the genus level (De Vantier et al. 2006; Balata et al. 2005; Wielgus et al. 2004) while algae grouped into morpho-functional groups (Littler 1980; Littler and Littler 1980; Steneck and Dethier 1994; Ateweberhan et al. 2006). A two-dimensional nMDS (non-metric multidimensional scaling), based on the centroids of replicate areas, was used for a graphical representation of the assemblages. Distances among centroids were obtained using principal coordinate axes from the original Bray–Curtis similarity index matrix.

The same model was used to perform the analysis of variance (ANOVAs) on total number of taxa, total percentage cover of organisms, percentage cover of the morphological forms of hermatypic corals (Veron 2000), soft corals, sponges and filamentous algae.

Results

The nMDS showed Site 3 separated from all the other sites. The dispersion of the centroids representing areas within each site were homogeneous with the exception of Site 4 where one area scattered far from the others (Fig. 3).

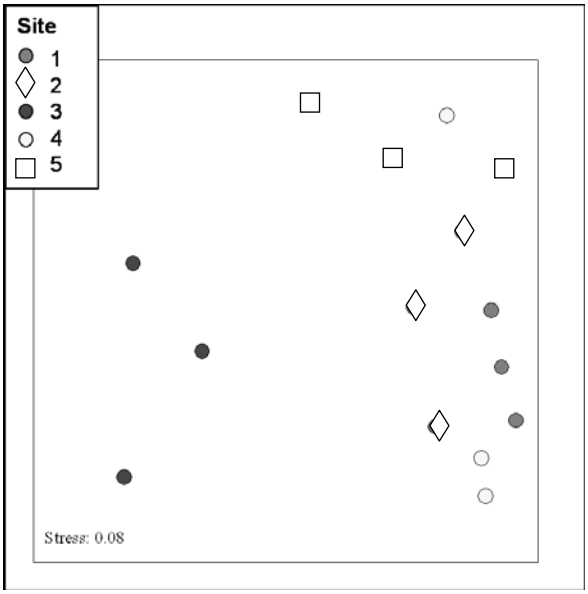

Figure 3: nMDS calculated on the cover values of benthic organisms for each Area

PERMANOVA showed significant differences in the structure of assemblages at both spatial scales investigated (Table 1).

ANOVA showed an analogous pattern, but branching corals showed significant differences only among sites while filamentous algae, sponges and the total number of taxa showed significant differences among areas (Table 2).

The mean percentage cover of all organisms was higher at Site 3 (30.15 ± 2.4 considering the mean among areas) than in the other sites with values between 11% and 16%. Filamentous algae were the most abundant organisms at all the sites with the exception of Site 3 where the percentage cover of massive corals was highest (Fig. 4).

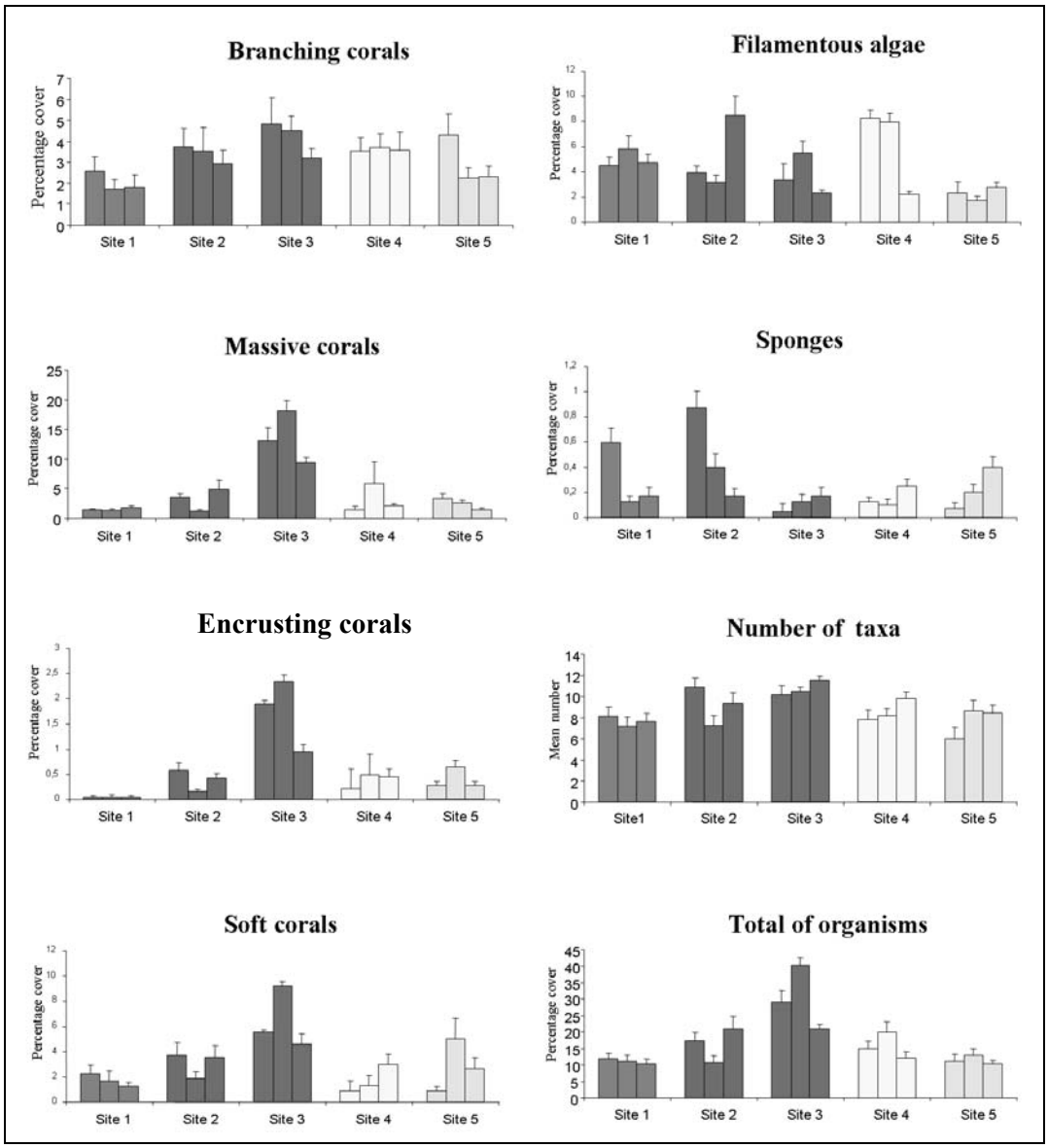

Figure 4: Distribution and abundances of the response variables used for ANOVA. Error bars indicate + SE, n = 20.

Table 1: PERMANOVA results. Significant values bold. (df: degrees of freedom; MS: mean squares; P(perm): P-values obtained using permutations; Pseudo-*F*: explanation in Materials and Methods; perm: n° of permutable units)

Source of variability	df	MS	Pseudo-*F*	P(perm)	perm
Site	4	33120.00	4.92	**0.0001**	15
Area (Site)	10	6727.20	3.35	**0.0001**	300
Residual	285	2007.00			
Total	299				

Table 2: ANOVA results. Significant values are in bold (* P < 0.05; ** P < 0.01; *** P < 0.001).

Source of variability	df	Branching corals		Massive corals		Encrousting corals		Soft corals		Filamentous algae		Sponges		Number of taxa		Total of organisms	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Site	4	2.70	**11.60*****	1476.54	**12.69*****	25.80	**10.35****	224.62	**4.36***	4.70	2.01	0.55	1.33	95.23	3.33	3614.40	**6.62****
Area (Sito)	10	0.23	0.41	116.33	**3.14*****	2.49	**3.98*****	51.49	**4.03*****	2.34	**4.92*****	0.41	**7.82*****	28.60	**2.18***	545.61	**4.96*****
Residual	285	0.57		37.08		0.63		12.78		0.48		0.05		13.11		109.98	
Total	299																
Cochran C test		C = 0.1205 (ns)		C = 0.1681 (P< 0.01)		C = 0.4010 (P<0.01)		C=0.2773 (P<0.01)		C=0.1207 (ns)		C=0.1364 (ns)		C = 0.1089 (ns)		C = 0.1681 (P < 0.01)	
Transf		ln(x+1)		none		none		none		ln (x+1)		ln (x+1)		none		none	

Discussion

Our findings indicate that the structure of the assemblages and the abundances of dominant taxa varied at the scales of tens and hundreds meters along the investigated coast.

Understanding the scales of natural variability of the response variables analysed is a fundamental requisite for the design and optimisation of any environmental sampling programme (Underwood 1991). As a first step of the environmental impact assessment based on a "Beyond BACI" design, the variability observed during this study will be taken into account and compared with the results of future sampling.

One of the studied sites (Site 1) showed a low cover of living organisms; therefore this site can be considered, from an ecological point of view, the most suitable for the construction of the Underwater Observatory. Patterns of distribution showed that Site 3 hosted the largest abundances of corals. This was likely due to spatial differences in the hydrodynamic conditions of the studied coast. Even if further investigations should be necessary to confirm this hypothesis, it is possible that recruitment and development of corals at Site 3 is enhanced by exposure to waves and currents greater than at the other sites.

History of previous disturbances that have occurred along this coast (storms, bleaching events and *Acanthaster planci* outbreaks) could explain low percentage cover of scleractinian corals and the relative high abundance of filamentous algae. However, the observation of numerous small colonies suggests beginning recovery of corals (authors personal observations).

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Coral reef conservation campaign in Brazil

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Abstract The Brazilian Ministry of the Environment launched the Coral Reef Conservation Campaign in 2001, understanding that public awareness is crucial to environmental conservation. The main objective of this campaign was to promote principles of environmentally friendly behavior based on the international "Leave No Trace" Campaign. In the first phase of the Campaign, managers of nine protected areas established in coral reef sites were invited to be partners. In 2005, with the financial support of the National Fish and Wildlife Foundation, environmental training courses were developed targeting the five most visited Brazilian beaches with coral reefs (Maracajau, João Pessoa, Porto de Galinhas, Tamandaré and Porto Seguro). Various outreach materials were prepared: posters, folders, textbooks, stickers, t-shirts, caps and DVD-videos. We also established partnerships with airlines and a public TV channel to broadcast the video, and supported its use at events, such as "Clean Up Day", in three beaches: Natal, Tamandaré and Maceió. In this paper, we describe the methodology, dissemination and partnership strategy used, and we discuss the results and evaluation of the awareness campaign.

Keywords: Coral reef conservation, Brazil

Introduction

Coral reefs are distributed over 3,000 km along the northeastern coast of Brazil, representing the only coral reef ecosystem in the South Atlantic (Maida and Ferreira 1997). Most of the reef building corals are endemic, forming structures not found in any other part of the world. About 18 million people live on the coastal zone of the Brazilian northeastern region. The health, well-being and subsistence of these coastal populations benefits directly from the environmental quality of the coastal ecosystems, especially of the coral reefs (Maida et al. 1997). Nevertheless, due to unsustainable use of resources over the years, several Brazilian reefs, mainly coastal ones, are showing increasing signs of stress. It is imperative that actions be taken to protect these ecosystems. Public awareness of coral function and value seems to be fundamental to achieving conservation.

The Brazilian Ministry of the Environment (MMA) launched the "Coral Reef Conservation Campaign.", which promotes environmentally aware behavior based on the international "Leave No Trace" Campaign. Ecotourism in Marine Protected Areas is considered the best option for making these areas financially self-sustainable. If, however, visitation is uncontrolled and careless, it can become a serious threat to the protection and conservation of local biodiversity. In this case, public awareness of the roles and importance of wild areas is a key step for environmental conservation. Building on this principle, the MMA decided to launch an awareness campaign directed to the visitors of these protected areas. The first phase of the Coral Reef Conservation Campaign was prepared by the Brazilian Ministry of the Environment and the Coastal Reefs Project in 2001/2002 (MMA, 2002). Its objective was to conserve coral reefs by reducing harvesting on reefs and controlling damage or destruction of the coralline structure. In 2004, the Brazilian Ministry of the Environment received a grant from the National Fish and

Wildlife Foundation to continue the Campaign in partnership with the Mater Natura Environmental Studies Institute to expand the campaign, showing the importance of coral reef protection to tourists and environmental agents, managers of protected areas, teachers and environmental volunteers. Activities included: (i) producing printed material; (ii) producing a video with coral reef information for distribution to tourist centers, airplanes, local community centers, and dive shops; (iii) launching a training program for managers of protected areas, teachers, tourist guides and volunteers; and (iv) evaluating the campaign.

Material and Methods

This project aimed to extend the original campaign "Leave No Trace" to coastal and marine zones, specifically for coral reefs, with the production of educational brochures and a DVD. As the primary goal of campaigns for the conservation of wild areas is the creation of behavioral guidelines for tourism and leisure activities in terrestrial areas, the campaign was followed by developing suitable guidelines to be applied to marine ecosystems in Brazil. Coral reefs were the first to be selected. A public awareness campaigns on the role and importance of the population was considered the principal measure to ensure protection of these ecosystems. The overall purpose was to create the most attractive products and a logo that identified the campaign (Fig 1).

Figure 1: Logo of the campaign

Three different types of campaign materials were designed (folder, poster and booklet, www.mma.gov.br.

In the second phase, activities were reinforced in five reef areas that were suffering extensive impacts due to coral collection, fishing, sedimentation, pollution and actions resulting from urban occupation and unplanned tourism. In 2004, the Coastal and Marine Division was created within the Brazilian Ministry of the Environment to become the focal point of the campaign. The training program was launched in the states of Rio Grande do Norte, Paraíba, Pernambuco and Bahia.

Activities were: DVD and new dissemination materials, a training program and the evaluation of the campaign, analyzing the courses and the outreach material through questionnaires and interviews with the participants of the courses. The training program was held in the beaches of Maracajau, João Pessoa, Porto de Galinhas, Tamandaré and Porto Seguro and consisted of at least 10 hours of lessons, including topics on coral reefs, the importance of conservation, Brazilian coastal protected areas, coral reef monitoring and techniques to minimize impacts on natural environments. The project evaluation report included four criteria and indicators: (1) participation rate: an indicator of the number of people interested in courses; (2) number of committed implementers and a description of participant's involvement in the campaign; (3) the level (presence/absence) of municipal endorsement of the program and the integration of the campaign in other community programs (total number of activities launched with the campaign); and (4) the attitude toward coral reef conservation among tourists. The tourists' evaluation was prepared by five environmental agents from the local community.

Results

The campaign began with the distribution of printed material to protected areas and, in the second phase, the final product of the project was to engage institutions and stakeholders in five areas highly endangered by intense tourism. The courses were attended by 180 persons and the outreach material distributed to schools, tourist centers, local communities, universities and municipalities (Fig 3).

Figure 3: Printed material of the campaign

A set of guidelines based on the major principles people should bear in mind when visiting reef areas, regardless of whether these areas are protected or not, was produced. These guidelines are: a) scuba diving can be the best way of exploring coral reefs, but divers should contact professional divers and managers of protected areas for information, and follow the protected area's rules when planning to visit the area; b) take care to drop anchor onto the sand, because when set onto the reefs, corals and other organisms are damaged, in addition to it being illegal; c) refrain from stepping or touching the coral, they are very fragile organisms; d) feeding fish is harmful to their health; e) when diving, use waterproof skin lotion; f) shells, corals and starfish provide shelter to other organisms, so should not be removed from their habitat; g) divers must maintain control of diving equipment so that it doesn't bump against the reef; h) avoid wearing fins in shallow waters because they can damage corals and other organisms and suspend sediments; i) move slowly when diving in order not to scare animals away; j) the law prohibits buying and selling handicrafts made from corals, so do not purchase these; k) try to obtain information on the tide times and cycles in order to avoid unexpected, potentially dangerous situations; l) do not use explosives, liquid bleach and other chemicals for fishing as these are extremely harmful to the reef environment and their use is prohibited by law; m) avoid using harpoons and fishgigs, as they scare animals away; n) when visiting a natural area, take the trash you produce back with you; and o) do not collect anything, make sure that the only things you take away from the reefs are memories and pictures (Fig. 4).

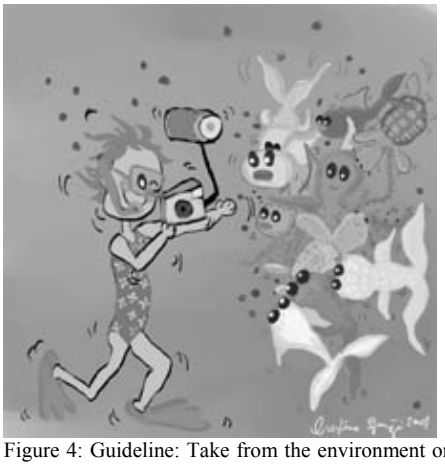
Figure 4: Guideline: Take from the environment only memories and pictures

The course evaluations ascertained that the activities and the outreach material were appropriate for communicating about coral reef ecology and the campaign for minimum impact conduct. It also provided an opportunity to discuss coral reef issues among the various local institutions, who suggested more courses and the creation of a coral reef network. The results of the campaign evaluation show that at some beaches, local agents gave lectures to the public, explained the issue in schools, carried out a Clean Up Day, and distributed posters in shops and restaurants and stickers and folders in boats. The evaluation of the attitude toward coral reef conservation among tourists resulted from 168 interviews in September 2007, to acquire information on visitor awareness and behavior towards coral reef areas in the five beaches of the project. Out of the 168 tourists interviewed, 144 said that they do not buy handicrafts made with coral and 99 do not feed fish when visiting a coral reef area. They chose a coral reef beach because of its beauty, some have marine parks and they could dive and see the coral reef ecosystem. This information shows that in some places there is concern for environmental protection. A case in point is the marine protected area of Recifes de Coral, in Maracajau beach. Its council created rules like limiting the number of boats that can use the area, established the presence of environmental guards and volunteers that explain the importance of coral reefs. In João Pessoa, a large campaign was carried out in the summer for tourists. In

Porto de Galinhas, the number of boats is limited and in Tamandaré, the Pernambuco Environmental Company carried out an environmental education program and set up an environmental library. In addition, we established a partnership with a Brazilian airline to show the coral reef DVD in the airplanes that fly to the northeast of Brazil and to support the Clean Up Day activities in three beaches: Natal, Tamandaré and Maceió. These specific events were organized by local NGOs: Oceânica, in Rio Grande do Norte, the Pernambuco Environmental Company in Tamandaré and the Mergulha Maceió dive shop in Maceió. These events attracted more than 200 volunteers. In Porto Seguro, we received support from the Coral Vivo Project, which developed a coral reef research project and used some campaign material to inform the tourists.

Discussion

In Brazil, some environmental education and awareness raising initiatives have been specifically developed for the coastal zone. Some of these are aimed at diving activities or at attempting to establish underwater interpretative trails. The distribution of information material in itself is not the only way to meet the broader objective of responsible conduct, but it has an important role in the comprehension of certain concepts. The basic principle behind an awareness raising campaign is to inform a group about the importance of the conservation and the minimum impact conduct. The training courses and the outreach material are a way to reinforce the conservation activities developed by some institutions in the coast. One possible means of evaluation is to hand out questionnaires to the visitors of the areas, before and after they receive the material, on their attitudes with regard to the environment or their knowledge of the fragility, value and function of the particular ecosystem. The Ministry of the Environment carried out a survey with the participants, tourists and environmental agents on the receptiveness of the Campaign and is also using the campaign in 2008 to disseminate the International Year of the Reef.

Acknowledgment

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Voluntary Standards as a Tool for Increasing the Sustainability of the Marine Recreation Industry and Improving MPA Effectiveness in Hawaii and Mesoamerica

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Abstract. Standards have a long history of improving service quality and safety in a wide range of industries. Successful businesses benefit from standards both by actively participating in the standardization process and by using standards as strategic market instruments. The Coral Reef Alliance (CORAL) recently completed a process in which marine recreation industry stakeholders in Hawaii and along the Mesoamerican Barrier Reef (MAR) worked in unprecedented collaborations to develop voluntary standards that will measurably improve and sustain environmental performance in scuba diving, snorkeling, boat operations, and marine life viewing. Engaging a standards committee comprised of representatives from marine recreation, conservation NGOs, tourism industry associations, marine recreation suppliers, marine park managers and government agencies, scientists, divers, local community groups, and traditional communities, CORAL is now testing the implementation of these standards and providing technical and financial support for locally based conservation initiatives. It is expected that this process will enhance adoption of standards throughout the marine recreation industry, increase industry support for marine protected areas (MPAs), and lead to the development of extensive conservation alliances, which enhance MPA effectiveness and improve the economic and environmental sustainability of marine recreation in Hawaii and Mesoamerica.

Key words: Standards, Sustainability, Tourism, Hawaii, Mesoamerica

Introduction

In areas of high-volume tourism, repeated direct contact with the reef poses an immediate threat. Hundreds of boat groundings and hundreds of thousands of tourist interactions each year reduce sections of coral reef to rubble. Human contact also reduces coral's ability to cope with stressors such as rising sea temperatures and diseases.

The development of voluntary standards for marine recreation operations provides detailed requirements for environmentally friendly and safety-conscious marine tourism businesses in the areas of diving, snorkeling, boating, and marine life viewing. The voluntary standards are crafted to be flexible and can be used by a variety of groups, including:

- Concerned tourists: to help them choose sustainable and safe marine excursions
- Marine tourism businesses: to evaluate and improve their own practices
- Non-governmental organizations and governments: as a basis for creating their own standards
- Bulk purchasers like cruise ships: to select sustainable and safe business partners

This paper offers two contrasting case studies of how voluntary standards development can be embraced, as experienced in Mesoamerica, or initially rejected, as seen in Maui, Hawaii, by stakeholders. Key lessons learned in standards development are identified to assist with future initiatives.In addition, unique challenges to widespread standards implementation are indentified. For example, the exponential growth of cruise ship tourism in recent years-carrying an estimated 6 million tourists to Mesoamerica in 2004 alone – presents a new impact that must be addressed in this region's overall marine conservation efforts. Ensuring cruise lines both support and contract with local tourism operators who implement the voluntary standards will be a critical test of long-term viability and efficacy of standards as a tool for resource managers.

Case Study -Mesoamerican Reef (MAR)

Material and Methods

CORALfacilitated a highly participatory process to develop the world's first Voluntary Standards for Marine Recreation in the MAR System—Scuba Diving Services, Snorkeling Services, and Recreational Boat Operations.Starting in early 2005, over 180 participants from across the four countries of Mesoamerica (Mexico, Belize, Guatemala and Honduras)representing marine tour operator associations, park managers and conservation NGOs, the cruise ship industry, scientists, concerned divers and individuals from the local communities worked together to develop and implement these voluntary standards andinitiate local and regional conservation projects throughout Mesoamerica.

The widely recognized due process practices defined in the Standards Engineering Society's standard SES-2, Model Standards Development Procedure, were followed in developing these voluntary standards. All drafts, comments, discussions and balloting were accomplished online simultaneously in Spanish and English.

Existing guidelines, preferred practices, and codes of conduct that have been developed by a number of organizations were considered for adoption, but were found to be insufficiently prescriptive and specific. Their focus was directed more towards tourist behavior rather than the conduct and responsibilities of the marine recreation operators and their staff.

Additionally, with respect to the development of the *Voluntary Standards for Recreational Scuba Diving Services*, the European Committee for Standardization's EN 14467:2004, Requirements for Recreational Diving Service Providers, was thoroughly reviewed. However, it was determined that too many additions and exceptions would have to be incorporated to address sustainable tourism issues, particularly regarding the environment, that are critical to application on the Mesoamerican Reef.

In July 2006, the Voluntary Standards were unanimously approved, and then published in May 2007. To date, there is widespread support for standards implementation in the region. With continued education and training on sustainable tourism and reef conservation issues, region-wide implementation is gradually becoming a reality.

Long-Term Assessment

Voluntary standards implementation and assessment consists of a three-part program: 1) a self-evaluation consisting of checklist and narrative progress reports of operator's own performance against the standards, 2) peer-to-peer evaluation across tourism operators using identical checklists and narrative evaluation, and 3) an anonymous third-party "secret shoppers" program to gain additional verification data on operator performance. Data gathered from this testing phase identifies challenges for widespread adoption of the standards, and informs the future direction of work in promoting sustainable marine recreation in each region.

Results

By early 2008, several businesses in Mexico and Belize have been assessed and are demonstrating implementation of standards and good practices. As proof of concept, three companies in Cozumel, Mexico, which collectively carry more than 75,000 tourists into the Cozumel National Marine Park each year, are rewriting company policy manuals to incorporate requirements from the marine recreation standards related to staff training, visitor management and briefings, and operation of vessels. **CORAL** acknowledges this as a significant indicator of early willingness to reassess the business-as-usual mentality in favor of reef (and ultimately, business) sustaining practices.

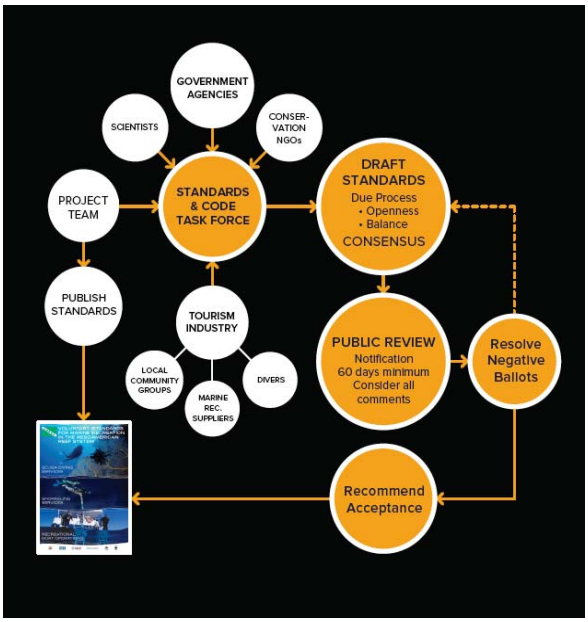

Figure 1: Successful process in Mesoamerica.

Case Study – Maui, Hawaii

Material and Methods

In 2006 CORAL was invited by the State of Hawaii Department of Land and Natural Resources, Division of Aquatic Resources to facilitate a voluntary standards development process on the Island of Maui. In contrast to marine tourism along the MAR, Maui's marine recreation landscape is highly politicized, heavily regulated, and relationships between resource managers and marine recreation operators are fractured. With the successful process experience in Mesoamerica, CORAL applied a similar approach to voluntary standards development on Maui.

Standards development on Maui temporarily stalled in early 2008 as a result of:

1. The State of Hawaii rewrote the Recreational Impacts to Reefs Local Action strategy to include language suggesting intent to regulate operator activity within marine protected areas based on the voluntary standards. This had a chilling effect on operator stakeholders who suddenly doubted whether the standards were truly voluntary.
2. The County of Maui threatened to close public beaches to all commercial recreational activity, raising concerns that a crackdown on recreational operators was imminent.
3. Following the sinking of a dive boat inMolokini Shoal Marine Life Conservation District, a $500,000 fine was levied against the owner of the boat.

Results

The multi-stakeholder voluntary standards development process on Maui broke down by late Summer 2008. The recreational diving community disengaged from a process where interests outside of the dive operators weighed-in on their business operation. As a result, a small faction of resistant dive tourism operators took it upon themselves to craft their own set of recreational diving guidelines. Not technically standards, these guidelines lack broad stakeholder inclusion and discussion. Though the Maui process stalled, a new multi-stakeholder standards development process was launched along the Kailua-Kona coast of the Big Island of Hawaii and shows early signs of buy-in and collaboration.

Discussion

If developed through an industry-led, consensus-driven process and adopted as day-to-day practice by marine tourism operators, voluntary marine recreation standards are attainable, affordable, and offer a means to dramatically reduce tourism's ecological footprint on coral reef protected areas. Some very clear lessons learned were identified from the MAR and Hawaii initiatives:

- Cultivate industry ownership of the voluntary standards
- Promote transparency throughout the process
- Facilitate locally-based partnerships and coordination to implement standards and support conservation initiatives
- Be prepared for challenges and delays based on fear of regulation
- Utilize incentives and momentum to promote implementation

Figure 2: Resistance issues expected in Hawaii.

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Hurricanes and corals in Southern Belize: from science to management and policy development

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Abstract. Although capacity building can potentially empower local communities with the ability to tackle socio-economic impacts of environmental change, evaluations of Integrated Coastal Zone Management (ICZM) performance can reveal limited interests in advancing community developments. This weakness has been apparent in Southern Belize ICZM, where there has been lack of communication between government ministries, local Non-Governmental Organisations (NGOs), and local communities. To address this, we undertook a capacity building exercise which involved all these sectors in Southern Belize, and developed a series of learning outcomes, linked to scientific goals. Surface areas of 523 individual coral specimens were measured, and recruitment dates were then modelled. We showed that hurricanes and severe storms limited the recruitment and survival of massive non-branching corals of the Mesoamerican barrier reef and on patch reefs near the Belize coast in the Caribbean, and suggest that marine park managers may need to assist coral recruitment in years where there are hurricanes or severe storms. From this scientific base, our project successfully engaged government and community-based partnerships in delivering realistic goals for the future of ICZM in the region.

Key words: tropical storms, cyclones, climate change, global warming, coral growth, fisheries, conch, lobster, snapper, community education, empowerment.

Introduction

Capacity building by engagement has been used in many communities where there are inherent and long-standing challenges to sustainability (Crabbe 2006), for example in Marine Protected Areas (MPAs) (Chircop 1998). While many, if not all, capacity-building programmes involve building competencies and empowerment in local communities, fewer involve policy makers or government officials (Mequanent and Taylor 2007). Capacity building is widely recognised as a central feature of Integrated Coastal Zone Management (ICZM) (Balgos 2005). However, although increased community capacity can potentially empower local communities with the ability to tackle socio-economic impacts of environmental change, evaluations of ICZM performance can reveal limited interests in advancing community developments. This weakness has been apparent in Southern Belize ICZM, where there has been lack of communication between government ministries, local Non-Governmental Organisations (NGOs), and local communities.

Since the Rio declaration on Environment and Development in 1992, the emphasis on capacity building and the transfer of technological knowledge and scientific understanding has encompassed four areas: legal and administrative capacity, financial capacity, technical capacity, and human resources capacity (Cicin-Sain and Knecht 1998). We wished to address the last two of these areas in Southern Belize by strengthening local research facilities and developing local communities' capacity to conserve and manage marine living resources (Wescott 2002). While developing capacity for coastal management has been achieved in the absence of government (Jorge 1997), we wished to involve the Belize government fisheries department in our study in order to facilitate future policy development.

This paper describes the development of a series of learning outcomes, which, for the first time, engaged government and community-based partnerships in

delivering realistic goals for the future of ICZM in the region. This paper examines three key areas specific to the Southern Belize MPAs:

1. Needs to address for the marine reserves
2. Tactics for leading, educating and supporting issues regarding sustainable development in Southern Belize
3. Development of policy issues for a better marine resource management zoning plan.

Material and Methods

There are two major coral reef areas in Southern Belize, the Sapodilla Cayes Marine Reserve (SCMR, a World Heritage Site), and the Port Honduras Marine Reserve (PHMR) (Fig. 1). The SCMR is a 125 km^2 reserve that has been under collaborative management between the Belize Fisheries Department and the Toledo Association for Sustainable Tourism and Empowerment (TASTE) since its declaration in 1996. The PHMR is a 414 km^2 reserve, and has been managed by the Toledo Institute for Development and Environment (TIDE) since its declaration in January 2000.

Figure 1: Coral reef Marine Reserves in Southern Belize. The Sapodilla Cayes Marine Reserve (SCMR), in dark grey shading to the left of the figure, comprises the southern end of the MesoAmerican Barrier Reef, and includes Seal Cay, Frank's Cay, Nicholas Cay, Hunting Cay and Lime Cay. The Port Honduras Marine Reserve (PHMR), in dark grey shading to the right of the figure, is close along the coast near Punta Gorda Town, and includes Frenchman's Cay and the Snake Cayes. Adapted from Crabbe et al. 2008a.

The key aims and objectives of the capacity building exercise were:

1. To increase the Belizean participants' capacity to lead, educate and support issues regarding sustainable development in Southern Belize; and
2. To promote networking throughout organizations managing marine resources, enhancing their power to collectively influence policy decisions in Southern Belize.

The capacity building team consisted of one officer from the Belize Fisheries Department, three senior officers from NGOs involved in managing Belize MPAs (TIDE, TASTE and Friends of Nature), and a Facilitator from the UK. These individuals were chosen because they had direct contact with both NGOs (Non-governmental organisations) and CBOs (Community-based organisations), and the government Fisheries Department, thus maximising exposure of capacity-building while keeping the numbers of participants within workable limits. Scientific measurements on coral reef colonies were conducted by the participants as described (Crabbe et al. 2008a). Daily meetings, lasting between 1-2 h,

took place on Lime Caye in the SCMR, Abalone Caye in the PHMR, and in Punta Gorda Town, over a 10-day period in August 2007 (Crabbe et al. 2008b). Discussions, led by the Facilitator, employed a modified nominal group technique (Sample 1984) to identify priorities related to personal action plans. Four rounds were employed; round one was based on the Delphi technique and further rounds on the nominal group technique approach (McCance et al. 2007). Specifically, after initial meetings which revolved around frank discussions on the interface been the Fisheries Department and MPA management by NGOs, each participant developed a personal action plan to facilitate and improve the sustainability of the MPAs in Southern Belize. There was repeated iteration of these plans between the participants, and the final production of a policy proposal for sustainable management of the marine reserves, Sapodilla Cayes and in Port Honduras.

Results

Our scientific work, described in Crabbe et al. 2008a, showed that for both the PHMR and the SCMR, there were significantly more non-branching massive corals recruited in non-hurricane years (mean 7.7 ± 5.6) than in hurricane years (mean 3.8 ± 2.9; p=0.011). When years with tropical storms are added to the years with hurricanes, there was significantly lowered coral recruitment (mean 4.7 ± 4.3) relative to non-storm or hurricane years (mean 7.4 ± 5.7; p=0.019).

The group then went on to develop personal action plans (Crabbe et al. 2009) and a set of learning outcomes by which the partnerships between government, NGOs and communities can improve ICZM. The learning outcomes are itemised below:

1. Management plans need to be passed into law. The involvement of the government fisheries officer as a partner is key to this outcome.
2. Zonation needs to be re-designated to balance stakeholders' wishes and evidence-based fisheries catches.
3. Participants will develop a community-based research programme.
4. Data need to be more accurate in the future.
5. Co-management plans between NGOs, communities and the Fisheries Department need to address the problem of illegal fishermen from outside Belize (Guatemala, Honduras) as well as from Belize.
6. Regular public meetings of stakeholders need to be fostered, as do regular education events.
7. Effectiveness of zoning needs to be quantified.
8. Alternative livelihoods for fishermen (e.g. in the tourist industry) needs to be fostered and maintained.
9. Tourists need to be monitored.
10. Effective management needs to be linked to the Belize economy. Fishing (conch, lobster, snapper, particularly in Southern Belize,) is an important part of the country's Gross Domestic Product (GDP).
11. NGOs need to link together.
12. Regular information sharing with all stakeholders, from the politicians to the local communities, needs to be maintained.

Discussion

Multiple stressors, hurricanes and bleaching, have caused significant disturbances to populations of coral recruits in Belize (Mumby 1999; Crabbe 2009). While we have not measured coral recruitment directly in this study, our modelling work has shown that hurricanes and severe storms have limited the recruitment and survival of massive non-branching corals on the fringing reefs near Discovery Bay (Crabbe et al. 2002; 2004), and here we show that the same is true for non-branching corals of the Mesoamerican barrier reef in the Sapodilla Cayes, and for corals on patch reefs in the Port Honduras Marine Reserve near the Belize coast in the Gulf of Honduras. What our work suggests is that marine park managers may need to assist coral recruitment and settlement in years where there are hurricanes or severe storms, by setting up coral nurseries (Forsman et al. 2006) and/or natural or artificial high rugosity substrate on the reef (Crabbe et al. 2008a).

Marine Reserves are an important tool in sustainable management of the Belizean coral reefs (Cho 2005; Williams and Polunin 2000). However, it is important that they share regulation, enforcement and conservation, underpinned by scientific research. We have already successfully transferred scientific expertise to Belizean participants for their use in generating scientific evidence to underpin future management and conservation decisions (Crabbe et al. 2009). Here, we have identified a number of key issues in marine reserve management in Belize than need to be addressed. Those issues, and the tactics employed by the participants in this project, will help ensure renewal of policy developments appropriate to the marine reserves in Belize.

The need for case studies in building integrated coastal management capacity has been powerfully made (Hills et al. 2006). Our study reinforced the idea that co-operative research improves capacity building and encourages innovative approaches to management, as has been found in northeastern USA, northwestern Europe, and coral reef MPAs (Johnson et al. 2007; Christie and White 2007). All participants felt that training and capacity building to key staff members are important, since this enables them to enhance their skills in the field and thus raise their standards to a certain level so as to perform better. They felt that this has been lacking in staff members of marine parks in Belize.

To date all 12 learning outcomes have been addressed by the local participants. A key positive outcome is that the NGOs TASTE, - TIDE and Friends of Nature have been incorporated into a single NGO, which spans four MPAs in Southern Belize. This means that areas between MPAs which were subject to illegal fishing activity will now be monitored and policed. The merger will enable assessment and enforcement across four different MPAs (Gladden Spit Marine Reserve, Laughing Bird National Park, SCMR and PHMR).

Developing ethical capacity for collaboration in the future (Coffin 2005) will need both resources and iteration over several years, and will involve a delicate balance of 'top-down' and 'grassroots' participation (McDuff 2001; Wescott 2002; Mow et al. 2007) to enable sustainable management. There were two issues that will need addressing in the future: the lack of involvement from the political arena, and 'local' Universities in the study. This paper shows that in Southern Belize conservation partnerships can come together with government to improve ICZM.

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Challenges for effective and sustainable co-managed Marine Protected Areas: a case study from the Comoros Islands

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Abstract. Co-managed MPAs must strike a difficult balance between development and conservation objectives and although terrestrial co-management initiatives for parks and reserves have been extensively studied, few evaluations have been conducted of co-managed MPAs. Mohéli Marine Park was the first MPA to be established in the Comoros in 2001. Initially regarded as a model for co-management of marine resources, the MPA is now operating at a vastly reduced capacity following an end to external funding sources. This study assessed current perceptions of local stakeholders of the MPA to evaluate the successes and challenges of the co-management approach. Concern was expressed for the lack of sustainability or alternative livelihoods; inequitable distribution of benefits; exclusion of women; continuing environmental threats and a concurrent lack of enforcement of regulations. MPA managers should carefully consider their options for sustainable sources of finance and lower-cost alternatives to ensure that management activities can continue beyond initial set-up funding. Donors potentially need to rethink policies for initiatives such as MPAs which may be more effectively implemented with a decentralized approach and long-term but consistent funding commitment over at least several years rather than current two- to three-year project cycles that generate visible but short-term results for the implementing team and donors and fail to deliver effective conservation and development outcomes for communities and their marine resources in the long-term.

Key words: Co-management, Mohéli, Comoros, Marine Protected Area, MPA

Introduction

The linkages between human and natural systems and the need for natural resource management approaches that address these linkages are widely recognized (e.g. Mascia et al. 2003). The success of Marine Protected Areas (MPAs) is intrinsically dependent on the behaviour of people in their use of coastal and marine resources (Bromley 1991; Francis et al. 2002; Francis and Torell 2004; McClanahan et al. 2006), thus MPA managers must strike a difficult balance between conservation and livelihood goals. Local stakeholders' perceptions of an MPA are an important indicator of its success and these perceptions will significantly influence their behaviour (Pollnac et al. 2001). Thus the co-management approach, whereby local stakeholders have considerable input into the setup and management of an MPA, has been widely recommended as a means to address these issues. Co-management systems have long been applied to terrestrial ecosystems but have been negatively criticized on their sustainability and appropriateness to local conditions. In a review of 23 such projects in Africa, Asia, and South America, for example, Wells et al. 1992 noted that 'measurable progress has been rare' and they concluded that all projects had failed to meet their stated objectives because 'the critical linkage between development and conservation is either missing or obscure'. However, few independent assessments of the sustainability and effectiveness of MPA co-management have been conducted.

Mohéli Marine Park (*Parc Marin de Mohéli;*Fig. 1), established on 19th April 2001 was initially regarded as a flagship example of MPA co-management, with a committee composed of representatives assembled from ten local villages, government, police and tourism operators (IUCN 2002; Gabrie 2003; Granek and Brown 2005). The MPA was created through the five-year United Nations Development Programme (UNDP) / Global Environment Facility (GEF) funded project 'Conservation of Biodiversity and Sustainable Development in the Federal Islamic Republic of the Comoros' (Project Biodiversity) (IUCN 2004). Currently, the MPA is operating at a vastly reduced capacity following the end of Project Biodiversity, and subsequent end in funding (Wells 2005; C3-Comores 2007, Hauzer et al. 2008). This study

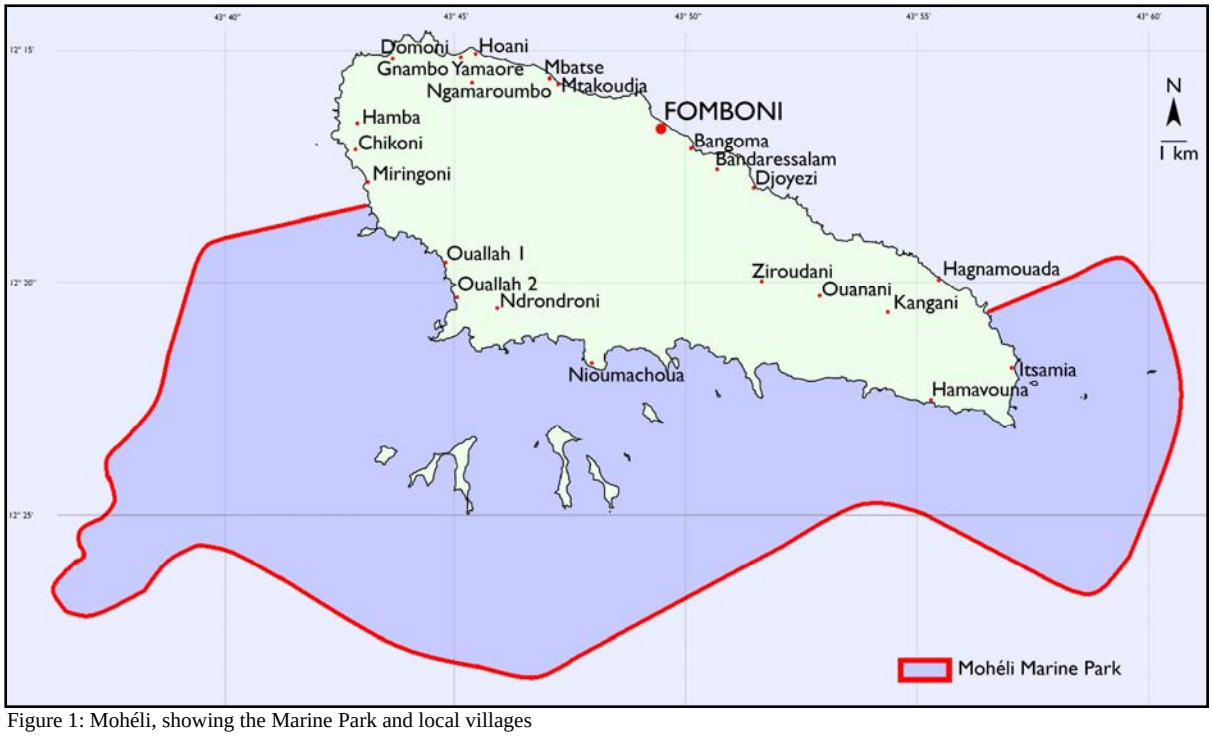

Figure 1: Mohéli, showing the Marine Park and local villages

involved an assessment of the perceptions of local stakeholders of Mohéli Marine Park to assess the challenges that have led to the demise of the once extremely promising co-managed MPA within five years of its establishment.

Material and Methods

Semi-structured interviews were conducted, following guidelines from Bunce et al. 2000, consisting of 12 questions based on six key parameters: (1) basic awareness, (2) value, (3) effectiveness, (4) environmental threats and solutions, (5) stakeholder roles and responsibilities and (6) future aspirations and expectations. The interview was designed to allow for open discussion in a focus group format and relevant follow-up questions were posed during each interview according to participants' responses to the key questions. Participants were selected to encompass the full diversity of stakeholders and male and female focus groups were strictly held separately to ensure that women would feel at ease in voicing their opinions. One male and one female focus group interview was carried out between 10th July 2006 and 20th August 2006 in each of the 10 villages of Mohéli Marine Park: Miringoni, Ouallah 1, Ouallah 2, Ndrondroni, Nioumachoua, Ouanani, Kangani, Ziroudani, Hamavouna, and Itsamia (Fig. 1).

Results and Discussion

Dialogue during the focus group interviews identified a number of positive and negative aspects of Mohéli Marine Park (Table 1). The 11 positive aspects identified corresponded fairly closely with the MPA's original objectives (Gabrie 2003), and participants in all villages believed that the MPA was important. However, the 18 negative aspects identified reveal significant shortfalls in the MPA's management regime.

Problems with sustainability at all levels were identified as the primary negative aspect of the MPA (Table 1). To ensure financial sustainability, Project Biodiversity laid the groundwork for a Biodiversity Trust Fund for the Comoros, including management of protected areas (Bayon 1999). However, a longer time-scale and greater level of capitalization than originally envisaged were required to set up the Fund (Wells 2005).

In the absence of the Trust Fund to cover base management costs, no contingency plan for sustainable funding and no lower-cost alternative for its management, Mohéli Marine Park's financial situation was uncertain following the end of Project Biodiversity in 2003. This was evident to local communities who remarked on the reductions in management effectiveness, activity and levels of enforcement after Project Biodiversity ended (Table 1), and led them to believe that they had been the victims of 'false promises' of the Project as illustrated by the following quote from the Ouallah 1 male focus group: *'Since Project Biodiversity finished, we now say that the words 'Marine Park' do not exist. There has never been follow-up to anything that was*

implemented during the project. With the project, we stopped all of the harmful activities that we were told to stop. However, we saw that we gained nothing, and now, bit-by-bit, we have started engaging in harmful activities again. Moreover, the management of the Marine Park now remains only in the hands of a few individuals, this is the reason that there is more damage occurring now than before, people have lost respect for the Marine Park. To make matters worse, the ecoguards were abandoned as soon as the project finances stopped.'

Table 1: Positive and negative aspects of Mohéli Marine Park identified by focus groups in approximate order of significance (Hauzer et al. 2008)

Positive aspects	Negative aspects
1. Environmental protection and a reduction in environmental destruction	1. Lack of sustainability
2. Increase in fish (size or number)	2. Lack of effective monitoring or enforcement
3. Prohibition of fishing gears	3. Lack of respect of Park personnel for official agreements
4. Increase in environmental consciousness	4. Poor management of equipment
5. Ecotourism	5. Absence of Park personnel
6. Increase in coral cover	6. No positive aspects
7. Exchange and increase in information through international interest	7. Prohibition of fishing gears
8. Infrastructure development	8. Lack of collaboration between Park, external organizations and villages associations
9. Reduction in unemployment	9. Insufficient environmental training, education, and awareness raising
10. Official permission for villages to protect their coastal zone	10. Lack of management of forestry activities
11. Presence of ecoguards	11. False promises of Project Biodiversity
	12. Absence of female participation
	13. Lack of benefits
	14. Lack of motivation
	15. No visible zoning of Park boundaries
	16. Inequitable distribution of benefits
	17. Environment in a worse state since the creation of the Park
	18. Lack of waste management

Lack of effective monitoring or enforcement ranked second for negative aspects of the MPA (Table 1). This issue was raised in eight villages, where respondents stated that a lack of permanent monitoring and enforcement had led to continued turtle poaching and destructive fishing practices. This in itself further de-motivated local communities as those abiding by the regulations were perceived to be the losers whilst perpetrators benefited from freely exploiting resources without due recrimination. The sheer scale, promises and community-conservation rhetoric of Project Biodiversity overwhelmed the local residents, raising their expectations in terms of anticipated benefits. Following the end of funding, communities were unwilling to accept the potential of low-cost, community-driven conservation initiatives, preferring to leave enforcement to the one or two salaried ecoguards who had been appointed by Project Biodiversity from their village. In this way, community motivation for natural resource management was, in some ways, reduced by the creation of Mohéli Marine Park as stakeholders became disillusioned by the lack of activity and tangible benefits following Project Biodiversity.

A lack of transparency in the management of Mohéli Marine Park and inequitable distribution of its benefits were major concerns voiced by local communities (Table 1). Stakeholders felt that benefits were being concentrated in the village of Nioumachoua, which housed the headquarters of the MPA or villages such as Itsamia that host more conspicuous marine attractions such as turtles. As illustrated by Mohéli Marine Park, employment in MPAs can be socially exclusive, in that the benefits accrue to a narrow subset of the local population. Often those employed are primarily from the best educated and motivated of their communities (one of the two senior staff of Mohéli Marine Park was not even Mohélian) rather than from the subsistence-stressed subpopulations that are the most likely to participate in illegal activity in the protected area (Barrett and Arcese 1995). This problem was recognized in 2001, when the gillnet and spear fishers of Nioumachoua expressed their dissatisfaction that Nioumachoua's alternative income-generating scheme (ecotourism facilities) had failed to provide them, the 'victims' of the MPA, with any benefits (Loupy 2001). These views regarding distribution of benefits were a root cause of the ubiquitous feelings of resentment towards the MPA. This dissatisfaction and distrust have clearly contributed to stakeholders' non-compliance with regulations and their unwillingness to actively participate in effective co-management.

It became evident through focus group interviews and discussion with MPA staff that Ndrondroni and Hamavouna were the most socially- and economically-marginalized villages and the also most excluded from its activities. Unsurprisingly, they were also the two villages most notorious for turtle poaching and a lack of compliance with MPA regulations, which was blamed on the communities' Anjoaunais origin (Boinali, pers. comm. 2006). Furthermore, as both villages have poorer infrastructure and services when compared to the other eight villages, they are also less likely to gain any direct benefits from tourism.

Generation of income through ecotourism was one of the key objectives of Mohéli Marine Park (Gabrie 2003) and was recognized by communities as a positive aspect (Table 1). However, tourist arrivals have declined since the MPA was established and communities complained that they were inadequately trained to host tourists and provide guides, accommodation and other services. Local capacity and infrastructure must be considerably improved for ecotourism to provide a significant alternative income on Mohéli (C3-Comores 2008). Although tourism is widely touted in co-management projects as having great potential to generate compensatory income, it has yet to deliver substantial sums as a result of the extreme variability in tourist revenues in response to political turmoil, exchange rate fluctuations and international economic conditions (Barrett and Arcese 1995).

Prohibitions on fishing gear (gillnetting, spearfishing, dynamiting and poisoning) were identified as a constraint by several communities (Table 1). The main concern was the reduction in catch as a result of restrictions, particularly during rough weather. There was also no consensus among communities on the actual effects of these regulations on fisheries yields. Without demonstrated fisheries-enhancement effects, Mohéli Marine Park will be unable to win over fishers who have lost income following gear prohibitions and area closures. Certain communities respected regulations but felt that their efforts were futile because fishers in other villages continued to use banned methods, benefiting from greater catches and a number of fishers felt that they had not received adequate compensation, such as alternative sources of income, alternative fishing gear and/ or training in new fishing techniques.

Project Biodiversity and other initiatives have, in the past, attempted to address these issues through the provision of motorized boats to fishers. However, logistical complications arose concerning the means for many fishers to share a single motorized boat and these projects have only caused conflicts in all villages that were involved in them. Some villages decided that the boat would not be exclusively used for fishing but also for tourism, transporting goods and passengers to other islands. Disputes also occurred if the boat or engine was damaged (Loupy 2001). This lack of positive conservation outcomes from incentive projects reoccurred in almost every village involved in the MPA and a lack of follow-up or formal evaluation of these alternative income generation schemes has made it impossible to learn lessons in order to improve such projects in the future (Malleret 2004).

Conclusion and Recommendations

It is clear that local stakeholders must be adequately informed of, consulted throughout and involved in the effective and sustainable management of MPAs. However, this work shows that in reality, there are pertinent and often overlooked challenges that must be overcome to implement co-management, particularly for large-scale projects. Although the Mohéli Marine Park project was a major, well-funded undertaking which employed international experts throughout the assessment and implementation stages, Mohéli's communities and their natural resources may now actually be worse off, not only materially but also through their altered perceptions of international donors and marine conservation.

The significant funding expended on the Mohéli Marine Park project and evident lack of sustainability after only five years of operation underscore the fact that large amounts of money do not necessarily ensure greater success in fulfilling management objectives. Perhaps more worrying are the unrealistic expectations conferred to local stakeholders from the beginning of the project which could only result in disappointment and disillusionment when promises could not be fulfilled. This now creates a conundrum for future work in Mohéli Marine Park because stakeholders are now unmotivated and wary of external interventions.

In the case of Mohéli Marine Park, tourism was the only large scale alternative livelihood option that was implemented to any extent. Perhaps it would have been more cost-effective to have conducted a detailed market analysis which would have revealed the need for a marketing strategy in tandem with the creation of tourism infrastructure. Now, although the buildings exist, without funding or the ability to market their tourism product, local communities will find it difficult to gain any revenue from tourism (C3-Comores 2008). Had sufficient consultation taken place with the fishers, the most relevant stakeholder group, alternative fishing gears and relevant training sessions could have been held to help offset losses in income due to area closures and gear restrictions. Inclusion of women in separate consultations would have revealed their significant roles in household income generation and provided options for developing their artisanal industries to offset losses in household income through restrictions in fishing activities. Such activities would be less reliant on external factors than tourism and thus potentially more sustainable in the long-term.

Donors need to pay more attention to long-term outcomes when considering a co-managed MPA project, and spreading available funding over five or ten years would potentially allow for more equitable distribution of benefits as management is adapted as

the MPA develops. This is particularly significant in cultures where effective and inclusive decision making processes work at a much longer time scale than the donor's project schedule. Experience has shown (Borrini-Feyerabend et al. 2004) that to achieve success, stakeholder-inclusive conservation projects often operate more effectively at small decentralized scales with small infusions of money over longer project terms. This smaller-scale approach to marine resource management on Mohéli may have allowed for a sustained management programme, better adapted to local capacity with reduced levels of conflict between the 10 villages. The negative correlation between the size of conservation projects and their effectiveness and success is supported by a report on GEF projects that determined that they had generally been a major failure except for the Small Grants Programme (Horta et al. 2002).

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Development of a strategy to guide the use of remotely sensed information in the management of coral reef environments

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Abstract. We have developed a strategy to guide the use of remotely sensed information in the management of coral reef environments in developing countries. The strategy includes development of a habitat map using satellite imagery, investigation of stakeholder receptivity to the habitat map, linking management issues to specific habitat types, and context-appropriate illustration of mapped data. The strategy was developed using IKONOS 4x4 m multi-spectral satellite imagery and interview data from dive operators, fishermen, and managers of Bunaken Island, Indonesia. The potential for this strategy to address coral reef management issues is wide-reaching and strongly depends on interaction between scientist and manager.

Key words: Remote Sensing, Local Knowledge, Coral Reef Management, IKONOS.

Introduction

Within the field of coral reef remote sensing, researchers have for many years been developing coral reef habitat maps (Riegl and Purkis 2005; Mumby et al. 2004; Andrefouet et al. 2003; Maeder et al. 2002). Each map contains a collection of habitat types, which are defined by their combined structural framework and biological composition (Veron 2000). These combined characteristics permit identification and delineation of the habitats within high-spatial satellite imagery (Mumby et al. 2004; Holden 1999; Knight et al. 1997). Within the last decade, these habitat maps have, in some places, contributed to coral reef management. This should be expected from habitat maps that delineate management-relevant information.

Until recently, however, there has been little interest in utilizing coral reef habitat maps for management in less-developed countries. There are many reasons for this: cost, available expertise, and general knowledge about the applicability of the information. In addition, there have been few opportunities for local communities to use such maps mainly because these communities have been removed from decision-making processes. In Indonesia, however, recent decentralization of the government has brought about increasing decision-making power to local levels, especially to small island communities (Nickerson and Olsen 2003; Erdmann et al. 2004). In the northern regions of Sulawesi, small island communities can now influence, more directly, management efforts (Erdmann et al. 2004).

If remote sensing-based coral reef habitat maps are to contribute to these management efforts, logically, it is necessary that the habitat maps illustrate information that is relevant to decision-makers. To this end, decision-maker knowledge must be integrated to the mapping procedure. In this study, we present a procedure that could be used to integrate decision-maker knowledge, to link this knowledge to the information in the habitat map, and to illustrate those habitats that are relevant to management. This procedure transforms habitat maps into 'management maps'.

Materials and Methods

This study involved the development of stages that together resulted in the development of management maps. To develop these stages, both the remote sensing and social contexts were considered. It was assumed that implementation of the stages would be facilitated by a remote sensing scientist. Therefore, we assume that the remote sensing scientist must engage in active research and collect and assess qualitative data. This means that the scientist is challenged to collect both physical and social science research and to integrate the information in an appropriate way.

Study Area

The study site for this research was Bunaken Island, which lies off the Northeast point of Sulawesi, Indonesia near the city of Manado. The island is surrounded by well-defined tropical coastlines, which

include a tidal flat, reef crest, reef slope, and reef wall. In some areas, the tidal flat extends up to 0.5 km from the shoreline. At the reef wall, the water depth drops down to the continental slope at 1840 metres. In the waters surrounding Bunaken Island, there are a minimum of 58 general and sub-genera of corals and approximately 2,000 species of fish (Mehta 2000).

According to the 2005 consensus there are three villages on the island of Bunaken: Alung Banua (population: 807), Tanjung Parigi (population: 610), and Bunaken (population: 2000). The people living in the villages are descendents of fishers who used Bunaken Island as a stopover location before heading out to sea. Dive operators also occupy the island and, at the time of this study, 15 are established and 11 are operating.

Bunaken Island was the first island in Bunaken National Park to be assigned a zonation system. The system includes three primary zones: core conservation zone, tourism use zone, and village use zone and is one of five islands situated within Bunaken National Park (BNP), which is 89,056-hectare in size.

In addition to the zonation plan, coral reef management activities include the placement of ecoreefs in shallow water coastal areas that have experienced considerable blast fishing. The coral reef crest environment nearby Alung Banoa Village and Fukui dive site now hosts over 60 ecoreefs that were provided by Seacology and implemented by NRM III personnel, local fishers and dive operators. An additional coral reef management activity that is gaining momentum is reef monitoring. Several dive operations have added a monitoring component to the Open Water, Rescue Diver, Dive Master, and Dive Instructor courses that involves quantitative assessments of health at various dive sites. Other management efforts include monitoring grouper species at identified Spawning Aggregation Sites (SPAG).

Research Methods

The research methods in this study are grounded in the broad methodological fields of remote sensing habitat map development and community participation in project development.

Qualitative data gathering tools included informal interviews, focus groups, and participant observation. The tools were selected using assistance from on-site researchers and from the Socioeconomic Manual for Coral Reef Management (AIMS, 2000). The qualitative data collection processes followed the principles laid down in Participatory Rural Appraisal (PRA) methodologies (Chambers and Guijt, 1998).

Based on the social context and the information available by the remotely-sensed habitat map the following four stages were identified: the first stage is to develop a habitat map; the second stage is to acquire stakeholder feedback to the habitat map; the third stage is to link the stakeholder feedback with habitat data; and, the fourth stage is to illustrate the linked information using stakeholder-identified cartographic elements. Each of these four stages was conducted in sequence and is described below.

Stage One: Development of a habitat map

First, two 4-metre multispectral and 1-metre panchromatic IKONOS satellite images of Bunaken Island were captured for July 7th, 2001 and June 6th, 2004. Both images were acquired with full 11-bit radiometric resolution and at 18-degrees off-nadir angles. Both images were acquired under conditions of light haze, < 20% cloud cover, and minimal surface water roughness. Image preprocessing included radiometric and geometric corrections, which were conducted by Space Imaging.

Field data included a library of ground observation points that were collected in both 2001 and 2004 for the purpose of sea-truthing the satellite images. A ground observation point represents one point on the ground, covering an area of 4 x 4 metres, from which geographical coordinates, water depth, substrate type and cover, and photographs were collected. Most points were selected randomly throughout all geomorphological zones (reef flat, reef crest, and reef slope) and all management zones (core, tourism, and general). Some points lie at half-metre intervals along transects that were placed over areas of interest, beginning at the shoreline and extend to the reef crest. Along transects, video surveys were conducted.

Initially, both images were georeferenced using GPS measured control points. The two images were then compared to each other. Areas of land, clouds, boats, and deep water outside the reef crest were then masked out. A basic atmospheric correction was applied to the imagery that involved sampling a large number of pixels from the 'deep water' area. A depth correction was then carried using Lyzenga's model.

For each image, over 600 ground observation points where then used to develop training areas, encompassing each of the six classes in all geomorphological zones. The training areas were input in a supervised maximum likelihood classifier. The final habitat map classes included coral (< 5m deep), coral (5-15 m deep), sparse seagrass, dense seagrass, silt, sand. The resulting habitat maps had overall classifications accuracies of 78% (2001) and 81% (2004).

Results

Stage Two: Investigation of stakeholder receptivity to the habitat map

The objective of stage two is to investigate stakeholder receptivity to the habitat map. Using informal interviews, focus group discussions, and participant observation techniques stakeholder receptivity to map content, map presentation, and map applicability to current and future management projects was investigated.

Qualitative data were collected in 2004 and the total number of respondents interviewed was 36, the total number of focus group discussions was 5, and participant observation was conducted regularly throughout the field period.

The following results were established. Generally, stakeholders agreed that a habitat map should contain no more than three to four habitats types. If the map contains more habitat types it is unlikely to be used. A habitat map should contain only those habitats that are relevant to each stakeholder group. In terms of map presentation, a habitat map should contain (1) the local names of the habitats, (2) land, deep water, and local references, and (3) standard cartographic elements such as a north arrow, scale, title, and legend that are written in the local language. In terms of map applicability, the habitat map should contain only those habitats for which known linkages between habitat and management issue are well know and established within the community.

Stage Three

The objective of stage three is to conduct an objective and systematic approach to linking management issues identified in stage two with habitat types identified in stage one. The researcher and research assistants deconstructed the feedback by using heir knowledge of the physical and social environments, and identified a degree of association between indicator and habitat. The 'degree of association' provides a means of quantitatively assessing the strength of association between indicator(s) that were identified by stakeholders and habitat(s) that were delineated in the IKONOS satellite imagery.

Results include a list of indicators, identified by stakeholders that describe the feature of interest such as a particular coral or fish species. The location and time period of interest was also identified. Results also include a list of habitats, which were delineated in the habitat map, that can be matched to the indicator. Then, a degree of association was calculated for all matches and results illustrate that 66% of the time, the habitat map contains habitat types that are relevant for addressing management questions.

Stage Four

A multitude of techniques is available for applying cartographic elements to a habitat map; however, the methods presented here focus on integrating general structure, reference features, habitat type illustration, and map format. Stakeholders created these categories: each category was identified as 'critical' to recognition and understanding of the mapped information. The categories were then used as a guide in the development of management maps.

Features of interest to each stakeholder group were highlighted or polygons were drawn around the feature. The legend of each map included the location of docks, villages, places of worship, and markets. A permanent feature in every map was the location of dive sites and the location of management zones. More specific features of interest, identified in stage two, were applied to each respective stakeholder map.

Discussion

It is important to note that throughout each field season for this research several key themes emerged.

(1) Tradeoff between cost and scale. For example, the fishermen on Bunaken Island are familiar with the spatial location of seagrass and, in many places, how the concentration of seagrass has changed through time. The cost of having this same information illustrated in a temporal management map at a fine spatial scale may far exceed what fishermen can contribute and may far outweigh the real use of the information. A more cost effective approach may involve acquisition of free satellite imagery, with coarse spatial resolution, investigation of seagrass concentration change using multiple images, and development of a temporal management map that illustrates only those regions with a considerable gain or loss in seagrass. This method provides the same information as is provided by a change detection analysis with IKONOS satellite imagery, however, the cost of the imagery is removed from the overall budget. Generally, in the future, it will be important to assess whether the cost of acquiring fine spatial scale information outweighs the cost of the imagery.

(2) Communication between scientist and manager. Considerable effort was made by the researcher to learn the local language and local terminology that is used to describe the coral reef environment. Though, at times, simply knowing the terms was not enough to capture the context and essence of the message communicated by the manager. To enhance communication, shared experiences (scientist-manager) are critical. Workshops, field trips, and joint presentations create a collaborative environment, one in which both scientist and manager can ask questions of each other and work together to target the same objective/interest. Field trips, for example, are an excellent opportunity to expose the manager to the

environment in which remotely-sensed field data are collected. Explanations on how the habitat data are collected and measured can be demonstrated, while in exchange the manager can ask questions and provide stories or explanations for certain locations or the state of specific portions of the coral reef environment.

(3) Community-based research approaches. An important key theme in this research is that community-based research approaches warrant attention, investigation, and inclusion in the strategy. To both capture and utilize the local processes of environmental management, it is critical to know how community-based research is employed, what information is deemed relevant and useful, and how the information is used. Without knowledge of these factors, it is nearly impossible to integrate foreign information or processes. However, identification of this information is challenged by communication and physical barriers. In some instances, meetings are closed to the public, to foreign researchers or students, or to personnel that are not directly involved in a project. During such instances, effort must be made to attend, to provide some skill or product in exchange for attendance. Because, it is often during such meetings, that decisions are made and the process of how these decisions are made is critical information.

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Monitoring and Evaluation: Lessons from Tubbataha Reef National Park and Coron Island Ancestral Domain, Philippines

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Abstract. Few efforts have been directed at evaluating the biophysical, socioeconomic and governance effectiveness of marine protected areas (MPAs) based on MPA objectives and management actions. This paper describes the process, indicators and lessons learned in developing monitoring and evaluation (M&E) programs for two MPAs in the Philippines: the Tubbataha Reef National Park and the Coron Island Ancestral Domain. The Tubbataha reefs, a World Heritage Site, covers 33,200 hectares of offshore reefs with exceptionally high biodiversity. Coron Island covers 22,284 hectares of ancestral (*Tagbanua* tribe) land and waters. The process to develop monitoring programs included: stakeholder consultations and consensus on indicators; creation of an monitoring team; data collection; information sharing; capacity building of monitoring team; and, institutionalization of management structure. Biophysical indicators measure the protection of marine species and habitats. Socio-economic indicators identified largely measure the economic status of the coastal communities in terms of household occupational structure. Governance indicators identified are mostly process indicators such as existence of a decision-making/management body and clearly defined enforcement procedures. Key lessons learned include: capacity building is a major bridge for overcoming technical difficulties in undertaking M&E functions; developing partnerships between local government and local stakeholders helps in accessing relevant information; and, a multi-disciplinary approach provides a comprehensive assessment for measuring the success of MPAs.

Key words: Coron Island Ancestral Domain, marine protected areas, monitoring, Philippines, socioeconomic, governance, Tubbataha Reef National Park

Introduction

In the Philippines, few efforts have been directed at monitoring and evaluation of marine protected areas (MPAs). The lack of adequate socioeconomic data and analysis of these information to provide concrete correlations between management actions and desired conservation outcomes of MPAs have not been well documented. This situation, in turn, weakens the support for establishing and maintaining MPAs. Without an effective monitoring and evaluation (M&E) program, tracking progress and providing feedback to MPA management cannot be achieved.

Monitoring and evaluation are distinct management functions, yet these are interactive and mutually supportive activities. M&E supports accountability in the use of resources and informed decision-making for corrective actions and to reinforce early signs of success. Various regulatory and management entities independently conduct M&E programs, which has resulted in duplication of efforts. Additionally, these entities often fail to present monitoring results in a form that is useful for shaping policy, resulting in the lack of a feedback loop that would permit periodic evaluation of the management effectiveness. Failure to effectively use information to undertake action and policy interventions leads to continued inefficient use of resources, and further deterioration of biodiversity in protected areas.

The purpose of this paper is to present the process in developing an M&E program to assess the effectiveness of management regimes for the Tubbataha Reef National Park (TRNP) and Coron Island Ancestral Domain (CIAD). These two MPAs are extremely high priority coral reef areas in Palawan Province, Philippines. The M&E indicators are described, as well as the lessons learned. This paper

forms part of the project of Conservation International–Philippines titled, 'Development of Management Monitoring and Evaluation Programs for Two Marine Protected Areas in Palawan, Philippines', funded by the U.S. National Fish and Wildlife Foundation.

Methods

Figure 1 presents the MPA location. Both protected areas are nationally and globally significant, not only because of their high levels of biodiversity, but also due to their high economic and social benefits. The TRNP covers 33,200 ha of offshore reefs in the midst of the Sulu Sea. It was established as a national marine park in 1988, and was designated a UNESCO World Heritage Site in 1993. Marine biodiversity is exceptionally high with at least 417 fish species, 372 coral species, 23 seabirds and eight species of marine mammals. These include a critically endangered sea turtle, an endangered marine mammal, seabird and sea turtle, and two vulnerable giant clams.

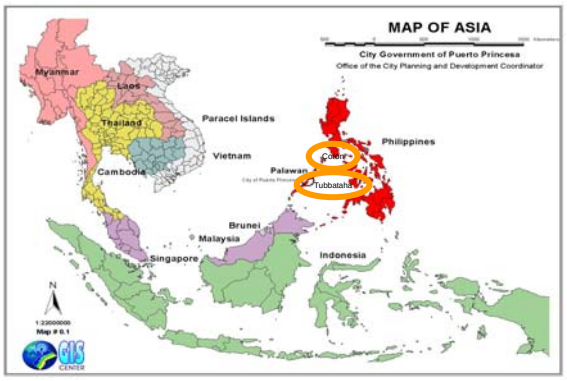

Figure 1: Location map of Tubbataha Reef National Park and Coron Island Ancestral Domain, Philippines.

The area of CIAD is within the Calamianes group of islands in northern Palawan Province, covering an area of 22,284 ha of ancestral land and waters. A unique marine biogeographic zone, CIAD harbors approximately 462 coral species, with at least 30 more new species recorded, and more than 700 species of fish. What makes this MPA unique is its direct management by indigenous peoples called *Tagbanwa*. A Certificate of Ancestral Domain Claim, a legal instrument given to indigenous groups to gain security of tenure over their ancestral land and waters, was awarded to the *Tagbanwa* tribe in June 1998.

Six key intricately-related and overlapping activities were undertaken to develop the M&E programs for the two MPA's. First, a series of multi-stakeholder consultations were undertaken to develop a consensus on identifying and selecting appropriate biophysical, socioeconomic and governance indicators. This involved participants from agencies and institutions that have mandate - in one form or another - in managing the two MPAs. This resulted in the formulation of an action plan for developing the M&E program strategy, including implementation and management arrangements. Second, the M&E team was created. This team was comprised of representatives from regulatory, management and research/academic bodies, other environmental groups, and indigenous peoples that were conducting monitoring programs or planning monitoring programs within the two protected areas. The team's main output was the design of the M&E program. Third, data collection was undertaken. It involved literature review, secondary analysis and primary data gathering. This was done as a collaborative endeavor among the participating institutions and stakeholder groups. Fourth, there was sharing of information collected. Primary data generated were analyzed and synthesized with the existing data and literature. This was followed by trainings as part of capacity building for the M&E team and stakeholders were conducted. Training exercises included topics on M&E concepts, rationale, socioeconomic survey methods, analysis and interpretation of results. Lastly, the institutionalization process for the implementation of the M&E program was initiated. This included the designing and agreement on the M&E proposed management structure.

Results

Three key result areas are presented. These relate to the following: (1) selection process for M&E indicators; (2) sample of datasets generated for indicators; and (3) rating for the relevant indicators.

Selection of the M&E indicators was based on the MPAs' management objectives contained in their respective plans.[1] The management objectives of both MPAs cover relevant biophysical, socioeconomic and governance dimensions (Table 1). Typical to most protected areas, these MPAs are trying to achieve an array of human and natural resources-related objectives. Based on these management objectives, coupled with intensive consultations with relevant stakeholder groups, the M&E indicators were selected. CIAD had 22 indicators (Table 2), while TRNP had 20 indicators (Table 3).

Table 1: Examples of biophysical, socioeconomic and governance objectives contained in management plans of Tubbataha Reef National Park and Coron Island Ancestral Domain.

Objectives	Tubbataha Reef Management Plan	Coron Ancestral Management Plan
Biophysical	To protect biological diversity and ecological processes	To protect and rehabilitate ecosystem function, communities,

[1] The TRNP already had a well established management plan. For the CIAD, this project assisted Coron management in drafting the Coron Ancestral Domain Sustainable Development and Protection Plan.

Objectives	Tubbataha Reef Management Plan	Coron Ancestral Management Plan
	from unnatural threats and human impacts.	habitat quality, species and gene pools.
Socioeconomic	To increase income potential from ecosystems targeted for conservation.	To stabilize or diversify household occupational and income structure through reduced marine resource dependency.
Governance	Ensure stakeholder participation and representation.	To ensure compatibility and enforceability between legal and local arrangements and improve the application of laws and regulations.

Table 2: Indicators for assessment of Coron Island Ancestral Domain management effectiveness.

Biophysical indicators	1. Focal species abundance 2. Focal species population structure 3. Composition and structure of the community 4. Type, level and return on fishing effort 5. Area showing signs of recovery 6. 6. Area under no or reduced human impact
Socioeconomic indicators	1. Local marine resource use patterns 2. Level of understanding of human impacts on resources 3. Household income distribution by source 4. Local values and beliefs about marine resources 5. Perceptions of local resource harvest 6. Household occupational structure 7. Number of tourists
Governance indicators	1. Level of resource use conflict 2. Existence and adequacy of enabling legislation 3. Local understanding of MPA rules and regulations 4. Level of stakeholder participation and satisfaction in management 5. Level of stakeholder participation in surveillance, monitoring and enforcement 6. Number of local legislations adopting national policies 7. Effective MOA among implementers of the ADSDPP

Table 3: Indicators for assessment of Tubbataha Reef National Park management effectiveness.

Biophysical indicators	1. Focal species abundance 2. Focal species population structure 3. Habitat distribution and complexity 4. Composition and structure of the community 5. Type, level and return on fishing effort 6. Water quality 7. Area showing signs of recovery 8. 8. Area under no or reduced human impact
Socioeconomic indicators	1. Local marine resource use patterns 2. Level of understanding of human impacts on resources 3. Perceptions of non-market and non-use values 4. Household income distribution by source 5. Number and nature of markets 6. Distribution of formal knowledge to community
Governance indicators	1. Level of resource use conflict 2. Existence of a decision-making and management body 3. Existence and adoption of management plan 4. Existence and adequacy of enabling legislation 5. Availability of and allocation for MPA administrative resources 6. Degree of interaction between managers and stakeholders 7. Clearly defined enforcement procedure 8. Degree of information dissemination to encourage stakeholder compliance

For the TRNP's eight biophysical indicators, the first four measure how much of the marine resources, biological diversity, individual species and habitat is protected. Species abundance (diversity) is defined as the number of individuals of a particular species found to occur within and outside the MPA. A focal species is an organism of ecological or human value that is of priority interest for management through the MPA. Data from the literature indicates that the seabirds' breeding populations have been maintained over the years (CI-Philippines, 2006).

Three examples for socioeconomic indicators are provided. The number of tourists engaged in diving has fluctuated over the years but shows an increase from about 500 tourists in 1989 to over 800 tourists in 2003 (Figure 2). Compared to other dive destinations, the trip to the TRNP is only feasible for about three months a year (March-May) due to weather conditions. The people of the Cagayancillo municipality, by virtue of political geography, are the TRNP's local stakeholders. Their dependence on marine resources (eg, fishing) has declined by over 50% since the establishment of the TRNP (Figure 3). The park's use values are largely generated through tourism (Figure 4). The TRNP is one of the most highly regarded diving destinations in the world.

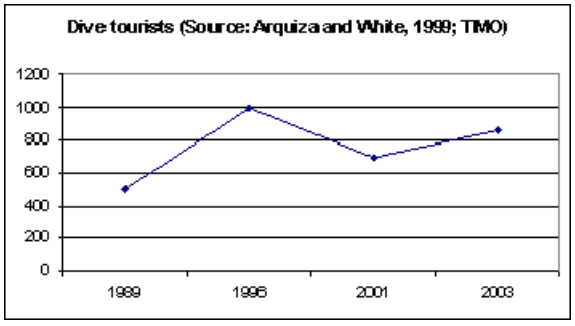

Figure 2: Local marine resource use patterns in terms of dive tourism in Tubbataha Reef National Park.

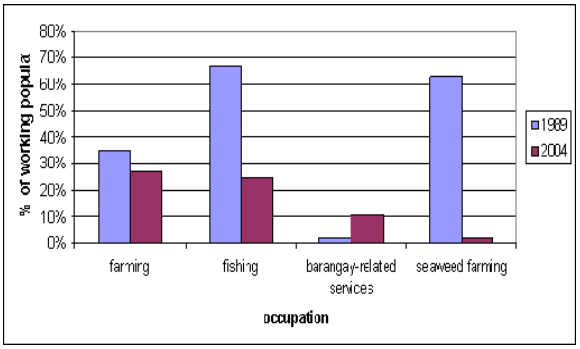

Figure 3: Household income distribution by source in Tubbataha Reef National Marine Park.

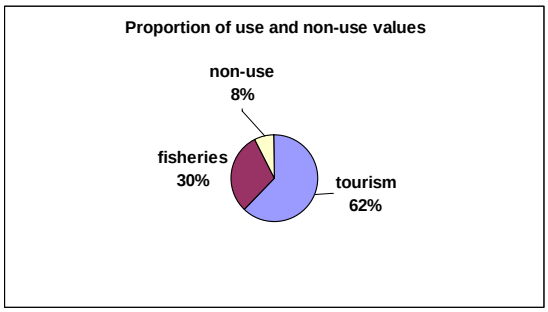

Figure 4: Perceptions of non-market and non-use values in Tubbataha Reef National Marine Park (Estimated value at US$ 64 million)

Two examples on the socioeconomic and governance indicators are provided in the case of CIAD. The stakeholders' perceptions of local resource harvest and opinions on level of resource use conflict are provided in Table 4 and Table 5, respectively. The people perceived that their yield from the fisheries is decreasing. Since smaller fishes are caught now, it implies biological overfishing. Conflicts appear to be escalating and appropriate measures are needed to resolve them. Legally, the municipal government largely operates through the Local Government Code of 1991 while the local *Tagbanuas* manage their ancestral domain through the Indigenous Peoples Rights Act of 1997. Although the municipal council did not dispute the evidence that the Calamianes Tagbanuas have held since time immemorial possession of CIAD areas, it claims also to have rights to the ancestral land and waters occupied by the indigenous peoples.

Table 4: Perceptions of local resource harvest by stakeholders at Coron Island Ancestral Domain.

Compared to 10 years ago	Response
Has the available target species changed?	Several species can no longer be seen like big eye scad, rabbit fishes, and siganids
What is the quantity of available target species?	A lot less- Before, fishing half a day yields 5 kg but now, fishing one day yields 2 kg. Before, 4 hours of fishing for fish yields 6-8 kg but now, zero.
Has the size of target Species changed?	Smaller fishes are caught now

Table 5: Opinions about level of resource use conflict by stakeholders at Coron Island Ancestral Domain.

Existing data	Data source
• Coron Municipal Council filed more than 14 municipal resolutions opposing the ancestral domain claim • Cited the interest of the burgeoning tourism industry, large-scale commercial fishing	Local NGO report
Primary data	**Data source**
• Small-scale fishers conflict among commercial fishers, tourist operators, local government	Key informant interview and stakeholder consultation

The suites of indicators are presented in summary format. Qualitative ratings are provided as follows: '+' for positive; '-' for negative rating; '0' for no change rating; and '?' for indeterminate rating due to insufficiency of information. As part of M&E process, the stakeholder groups and representatives of site M&E teams agreed on the above ratings and applied them in their preliminary assessments of their respective MPAs. This was undertaken during several stakeholder consultation workshops where stakeholder participants discussed and agreed on final appraisal of each indicator against baseline data

The biophysical objective in TRNP is 'to protect biological diversity and ecological processes from unnatural threats and human impacts'. Since most of the summary ratings for biophysical indicators are positive, it implies that the above objective has been largely met (Table 6). Except for 'perceptions of non-market and non-use values' which is rated indeterminate due to limited information, the rest of the socioeonomic indicators are all positive (Table 7).

Table 6: Summary rating for biophysical indicator on focal species abundance and diversity in Tubbataha Reef National Park.

Appraisal Against Baselines		Remarks
Seabirds:	Diversity +	Monitor breeding species only
	Abundance +	
Turtles:	Diversity 0	No data to determine abundance
Cetaceans:	Diversity ?	Cannot directly attribute to management effectiveness; 2004 data of TRNMP set as baseline.
Fishes:	Diversity +	1997 data of WWF set as baseline.
	Biomass +	
	Density +	
Indicator Fish:	Biomass +	Pomacentridae (negative)
	Density +	
Top Predators:	?	No sufficient data; Use 2005 data as baseline.

Table 7: Summary rating for socioeconomic indicators in Tubbataha Reef National Park.

Socioeconomic Indicators	Appraisal	Remarks
1. Local marine resource use patterns	+	Adapt 1986 baseline data
2. Level of understanding of human impacts on resources	+	Adapt 2004 baseline data
3. Perceptions of non-market and non-use values	?	Adapt 2004 baseline data; Uncertain trend
4. Household income distribution by source	+	There was diversification of income source and an increase in per capita
5. Number and nature of markets	+	Cagayancillo benefited from dive fees; stable price of seaweed
6. Distribution of formal knowledge to community	+	Need to examine impact of IEC materials; need to diversity IEC media (eg;TV, radio)

In the case of governance indicators, all are rated positively, except the indicator on 'availability and allocation for TRNP administrative resources' (Table 8).

Table 8: Summary rating for governance indicators in Tubbataha Reef National Park.

Governance Indicators	Appraisal	Remarks
1. Level of resource use conflict	+	There is positive effort towards reduced conflict (local fishers); emerging conflict between energy and fisheries
2. Existence of a decision making management body	+	Baseline data from Tubbataha Management Office, 2002
3. Existence and adoption of a management plan	+	
4. Existence and adequacy Of enabling legislation	+	
5. Availability and allocation for TRNMP administrative resources	-	Funds were adequate due to external funding through UNDP-GEF 2000-2004; Funds utilized in 2002 were from the dive fee collection
6. Degree of interaction between managers and stakeholders	+	
7. Clearly defined enforcement procedures	+	
8. Degree of information dissemination to encourage stakeholder compliance	+	Use radio for information dissemination.

Discussion

Developing and sustaining the operations of M&E programs for MPAs are difficult endeavors. This component of MPA management is not as well developed, when compared to other established components, such as information and education campaigns or law enforcement.

Attempts were made to come up with quantitative measures of success, which are beyond anecdotal evidences. Some substantial insights may be generated from this study. For TRNP, many of the indicators are rated positive based on trend or compared to an agreed baseline. Particular attention, however, must also be given to the allocation of financial and administrative resources.

In the case of CIAD, the fishery resources is apparently dwindling. If more people perceive that target species is decreasing, then this is not favorable and implies that more effort needs to be directed at determining and addressing the cause of this declining trend.

Several lessons were also learned in the course of developing the M&E programs for these MPAs. First, active involvement of the local government units is crucial for effective MPA management. Secondly, capacity building for the management staff and participating agencies is a major bridge for overcoming technical difficulties in undertaking M&E functions. Linking with local researchers and academic institutions is important. Thirdly, developing partnerships between the local government and the local stakeholders helps in accessing relevant information. Lastly, a multi-disciplinary approach, utilizing relevant mix of indicators, provides a more complete assessment for measuring the success of MPAs.

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Green Fins-Thailand: Coral Reef Conservation in the Face of a Tourism Epidemic

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Abstract. In Thailand, reefs along Andaman and Thai Gulf coast cover 160 square kilometers. Only sixteen sites are designated National Parks. Over 670 dive, snorkel tour and boat operators bring an estimated million guests to visit primary dive sites during the six month high season each year. Currently, there are many problems contributing to reef destruction. The Government lacks infrastructure planning for the growing tourism industry and promotes reef tourism without conservation and resource management. National Parks possess insufficient human resources. Dive and tour operators lack an understanding of reef ecology and use the natural resource for business only. The popularity of reef tourism is ever increasing and this lack of management is causing devastation on the reefs of Thailand. The Green Fins mission is to protect and conserve coral reefs by establishing and implementing environmental-friendly guidelines for divers. We actively seek out dive operators, introduce our program, educate and encourage operators to become members. We also arrange for individual members to participate in activities such as cleanups, reef monitoring, or exhibitions. Members receive certificates and materials to promote good diving practice. To renew membership, operators are assessed yearly based on Green Fins Code of Conduct criteria. We use this network to promote sustainable dive and snorkel tourism industry. We hope that in the future, tourists will begin to ask operators whether they are Green Fins members and choose to dive with Green Fins friendly operators. In this way, the Green Fins Program will establish a network of active conservationist divers and snorkels, spreading the word quickly and being an effective voice for positive change in the dive and snorkel tourism industries. We hope that someday, our methods will be applicable to other heavily toured nations facing similar problems.

Key words: Environmental-friendly, Sustainable, Network, Monitoring, Awareness

Introduction

Currently, there are many causes that contribute to coral reef destruction. The rapidly growing tourism industry has a negative impact on the reefs. That is why the Green Fins Program has been established in four countries, namely Thailand, Indonesia, Malaysia and the Philippines by the Coordinating Body on the Seas of East Asia (COBSEA). COBSEA is one of UNEP's Regional Seas Programs. In Thailand, Green Fins is implemented by the Phuket Marine Biological Center (PMBC), Department of Marine and Coastal Resources.

Methods

The Green Fins mission is to protect and conserve coral reefs by establishing and implementing environmental-friendly guidelines for divers. We actively seek out dive operators, introduce our program, educate and encourage operators to become members. Members receive certificates and materials to promote good diving practice. To renew membership, operators are assessed yearly based on Green Fins Code of Conduct criteria which consist of environmentally friendly standards for dive operator. We use this network to promote sustainable dive and snorkel tourism industry.

Green Fins Thailand has also developed a Reef Watch method to encourage divers and operators to help monitor their reefs. It is a one-dive-one survey method to record the health of coral reef that even snorkelers are able to practice. In addition, dive operators may provide this program to customers as a form of educational awareness as opposed to purely leisure. Data can be uploaded online via the website www.greenfins-thailand.org which will be input into a National Database. We promote IYOR activities by encouraging Tour Operator Members to adopt a reef in which members can choose different dive sites to monitor with a minimum of 2 data entry per year. We aim to have members attend these activities in all areas nationwide so that reefs and dive sites are monitored as much as possible.

Results

Currently we have 90 Operator Members and 302 Individual Members mainly from 6 provinces along Andaman Coast without recruiting for new members. We also have 211 Reef Watch data forms submitted by members. We try to work closely with them to actively promote Green Fins for sustainable diving tourism industry. We also arrange for operator members and individual members to participate in activities and encourage Operator Members to organize their own activities with tourist and local communities. Activities that Green Fins organize to raise awareness are

- Exhibition at Thailand Travel & Dive Expo
- Adopt-A-Reef, Adopt-A-Beach program
- Photo Contest topic on "Marine Litter the Sea and the Coast"
- Recycle Art Workshop from garbage washed ashore
- Organize International Coastal Clean Up
- Organize the IYOR meeting with Dive Operators, and divers

Discussion

We primarily work in management, monitoring and education areas. Assessment method could help select the green dive operator through the continued close contact with members. In the long run, Green Fins will become recognized as a standard to certify green dive operators. Raising awareness and education is also essential for people to understand why the guideline are applicable. By doing so, Green Fins needs more funding to support volunteers, to help coordinate with existing members, and to continue to recruit new members and expand into other dive location sites. Green Fins-Thailand can also be further developed for snorkel tour operators, and for raising the awareness of local people. All dive sites need full time attendance from coordinator, with a long term establishment of voluntary programs in order to see satisfactory results.

Conclusion

For sustainability of the Green Fins program, we are in a process of registering Green Fins to become an Association in order to expand already existing projects within the area, and raise funds from private sector groups to support a full time coordinator. We hope that in the future, tourists will begin to ask operators whether they are Green Fins members and choose to dive with Green Fins' friendly operators. In this way, the Green Fins Program will establish a network of active conservationist divers and snorkels, spreading the word quickly and being an effective voice for positive change in the dive and snorkel tourism industry. We hope that someday, our methods will be applicable to other highly toured nations facing similar problems.

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Restoration of threatened *Acropora cervicornis* corals: intraspecific variation as a factor in mortality, growth, and self-attachment

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Abstract. The potential of farming *Acropora cervicornis* corals to generate material for restoring the species to Caribbean reefs was investigated. Coral colonies from both high and low energy areas were sampled, to determine the relative importance of intraspecific variability and source environment on mortality, growth, branching density, and self-attachment. Isolated *Acropora cervicornis* thickets, were sampled, half from the reef front and half from the back reef. Each thicket sampled was assumed to be of a single coral genotype. Replicate fragments were sampled from each thicket and transplanted to wire frames located in shallow back reef areas <2m deep, on five reefs in the La Parguera reef system, Puerto Rico. Results at one year indicate that coral genotype is a highly significant factor for all of the variables studied, with up to a six-fold difference in relative growth based on genotype alone. Back reef and reef front populations of *A. cervicornis* corals differed significantly in relative growth, branch diameter, and attachment ability even after growing together for one year, suggesting a genetic basis to morphology and adaptation to their original environment. Frame culture was an effective method to produce second generation stock, with annual growth rates often over ten fold.

Key words: *Acropora cervicornis*, coral restoration, coral farming, coral gardening.

Introduction

Acropora cervicornis is composed of low-diversity, predominantly clonal populations of corals maintained by asexual fragmentation with clones shown to span distances as great as 30-50 m (Bothwell 1981; Neigel and Avise 1983). The clonal nature of the species has major implications for the restoration of this threatened Caribbean coral (Bowden-Kerby 2001a; Bowden-Kerby et al. 2005).

While remnant *A. cervicornis* populations may be predominantly composed of a single genotype and thus unable to effectively reproduce sexually (Bowden-Kerby 2001a), the fact that the species easily fragments to create clonal populations creates the potential for developing low-tech restoration methods modeled after and accelerating these natural fragmentation processes (Bowden-Kerby 2001a, 2001b). Alternatively, the highly clonal nature of the species might also present unforeseen challenges, particularly for this "generalist" species which occurs over wide environmental gradients, as each genotype may be highly adapted to a narrow range of environmental tolerances. It can be assumed that a particularly abundant genotype would be well-adapted to the set of environmental conditions where it occurs and that long-term selective processes would eliminate maladapted coral strains over time while favoring particular genotypes. If the survival or growth of coral transplants is strongly related to the original environment of the corals, closely matching the source environment of the corals with that of the transplant site would be important to long-term success.

This study focused on providing information on the relative importance of intraspecific differences in the mortality, growth, morphology, and attachment of *Acropora cervicornis* and *A. prolifera,* the hybrid between *A. cervicornis* and *A. palmata*.

The major questions were:

1. Are there significant differences within a coral species in mortality, growth, branching or attachment?
2. Are differences related to the origin of the coral: low-energy back reef vs. high-energy reef front?
3. Are transplantation site differences more or less important than interspecific differences?
4. Is the culture of corals on wire frames an effective method for producing second generation coral fragments for use in restoration work?

Material and Methods

For the purposes of this study, each isolated coral thicket sampled from a distinct reef was assumed to be a unique genotype, even though somatic mutation

could potentially occur within a single coral colony, causing diverse responses within samples of a particular coral. Zooxanthellae strains were also assumed identical for all replicate fragments of a specific coral, but differences between fragments could potentially arise by incorporation of new algal strains. To help control for these unlikely factors, 30 fragments were used per genotype, six replicate fragments at each of five sites.

The hybrid coral *A. prolifera* was treated as a distinct species. For each of the two species, eight thickets were selected, four from back reef areas and four from reef fronts. Morphological differences were clearly observed between the two populations of each species, and so reef front and back reef populations of each species are referred to as morphotypes. The 16 genotypes were obtained from reefs within the La Parguera reef system. Samples spanned 6 km and with two exceptions each sample reef was separated from other reefs by wide channels 10-25 m deep. Fragments were sampled from within a single, large, distinct coral colony or from a tight group of adjacent colonies (thicket) widely isolated from other corals of the species.

Frames for supporting coral fragments were constructed by bending 0.5 m x 1 m pieces of vinyl-coated 2.5 x 5 cm wire mesh into A-shaped frames, one meter long and standing about 25 cm high. Unbranched 8-12 cm apical coral fragments were attached to the frames with 10 cm plastic cable-ties, color-coded to denote replicate fragments of each genotype. Each fragment was secured at a mesh junction so that frame overgrowth could occur in all four directions along the wire. Separate frames were used for each species and for back reef and reef front corals, so there were four frames per site. All frames were located directly adjacent to one another on the sand substratum, randomly ordered and within 1-3 m of each other (split-plot design). The frames were weighted with short segments of heavy metal bars (Bowden-Kerby 2001a).

For the ANOVA analyses, mortality and relative growth means included data from all branches of each coral genotype. To minimize the hidden effects of breakage and mortality on branching, a branching density for each genotype was calculated based on half of the branches (3 out of 6), those with the highest relative growth per site. Branch density index was calculated as the length of the main branch in mm + 2 x length secondary branches + 3 x length tertiary branches ÷ total length of branches x 100. For overgrowth (attachment of the fragments to the frames), means were calculated based on the three maximum overgrowths per genotype per site, effectively eliminating branches or branch bases that had died.

For data presentation, each coral genotype was assigned a letter code: 'B' for back reef, 'F' for reef front, 'c' for *A. cervicornis*, 'p' for *A. prolifera.* Thus, Bc = back reef *A. cervicornis*, Fc = reef front *A. cervicornis*, Bp = back reef *A. prolifera*, Fp = reef front *A. prolifera*. Each genotype was also assigned a number from 1-4, with the number assigned based on the mean relative growth rates in descending order.

Results

Figure 1 shows mean mortality after one year for each coral genotype by site of origin. Comparing the genotypes in a randomized complete block design ANOVA, arcsine transformed data showed significant differences in mortality among reef front coral genotypes for both *A. cervicornis* ($p<0.0005$) and *A. prolifera* ($p<0.025$), however the ANOVA showed no significant differences in mortality among back reef genotypes of either *Acropora* species ($p<0.25$ in both cases).

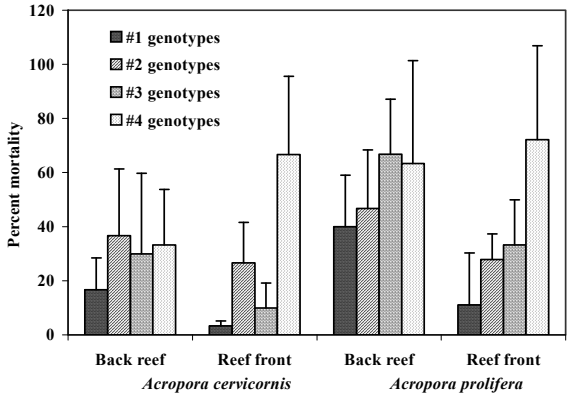

Figure 1: Mean percent mortality +SD of *Acropora* genotypes grown together for one year (n=30 per genotype, 5 sites x 6 branches; 3 sites for reef front *A. prolifera*. Genotypes are ordered based on relative growth: #1 with the highest, #4 the lowest.

Mean relative growth after one year is summarized in Figure 2. The results indicate the rapid growth of the various genotypes of each *Acropora* morphotype, ranging from a ten to twenty fold increase in branch lengths for *A. cervicornis* and from six to over thirty-fold increase over the year for *A. prolifera*. Two-way ANOVA comparisons indicate significant differences in relative growth among genotypes within coral morphotypes with transplant site as a factor (Bc: $p<0.025$, Fc: $p<0.025$, and Bp: $p<0.05$), except for genotypes of reef front *A. prolifera* (Fp: $p<0.50$). This lack of significance is likely due to smaller sample size, as only two sites included this morphotype due to a shortage of coral material.

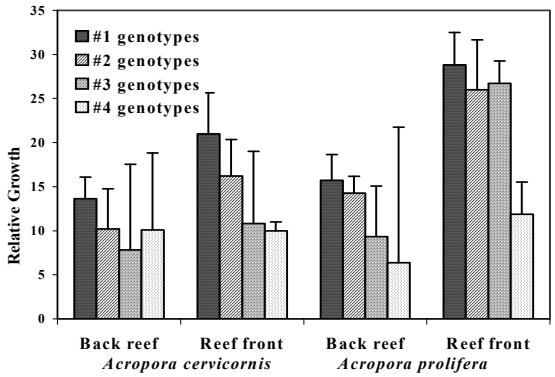

Figure 2: Mean relative growth based on maximum branch per site per coral genotype +SD (n = 4: 1 branch per coral genotype x 4 sites, 2 sites for reef front *A. prolifera*).

Figure 3 exemplifies comparisons that included all six branches per genotype per site. ANOVA comparing genotype means gives highly significant results for all but the Fp morphotype. (Bc: $p < 0.00001$, Fc: $p < 0.0001$, Bp: $p < 0.035$, Fp: $p < 0.3$). An interesting genotype-specific ordering of growth was observed for all four coral morphotypes, with particular genotypes growing fastest or slowest nearly everywhere, regardless of transplantation location (Fig. 3). This figure also shows the poor growth at the Media Luna offshore site due to extensive breakage. As breakage was considered a nuisance variable to the experimental questions, this site was excluded from the analysis. Without exception, the mortality ranking (high to low) of each genotype was the same as their relative growth ranking. This was the case when analysis included dead, broken, and lost branches, as well as when analysis was based only on the maximum branch per genotype per site.

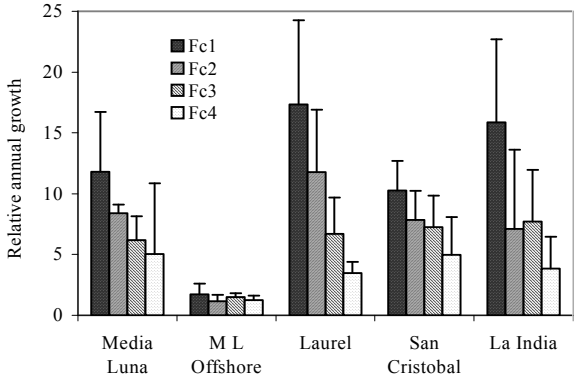

Figure 3: Site specific relative annual growth + SD of four genotypes of the reef front morphotype of *Acropora cervicornis* grown together on frames at five back reef locations. Each pattern represents a particular genotype (n = 6 branches per location).

Branch density data (Fig. 4) indicates that *Acropora prolifera* overall had a branch density index more than twice that of *A. cervicornis*. Comparisons between back reef and reef front morphotypes demonstrated no significant morphotype-specific differences in branching density within each species (paired *t*-tests: *A. cervicornis,* p=0.32; *A. prolifera*, p=0.39). Comparisons within each morphotype of the four genotypes indicated differences in branch density, confirmed to be statistically significant by randomized complete block ANOVA (Bc: $p<0.003$, Fc $p<0.005$, Bp: $p<0.025$, Fp: $p<0.05$). However, site was sometimes more significant than genotype in influencing branching (Bc: $p<0.005$, Fc $p<0.0005$, Bp: $p<0.0025$, Fp: $p<0.12$).

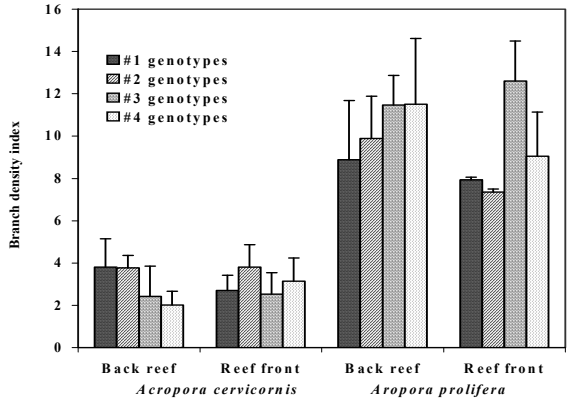

Figure 4: Mean relative branching +SD of *Acropora* genotypes grown together for one year in the back reef (n = 12: 3 branches x 4 sites; 2 sites for reef front *A. prolifera*).

Figure 5 shows mean overgrowth for each genotype of the four *Acropora* morphotypes, indicating a genetic basis for faster overgrowth of reef front corals, plus a generally positive relationship between relative growth and overgrowth. Randomized complete block design ANOVA of the data at one year gives statistically significant differences between genotypes for all morphotypes except for back reef *A. cervicornis* (Fa: $p<0.013$, Bp: $p<0.03$, Fp: $p<0.05$) (Bc: $p<0.38$.)

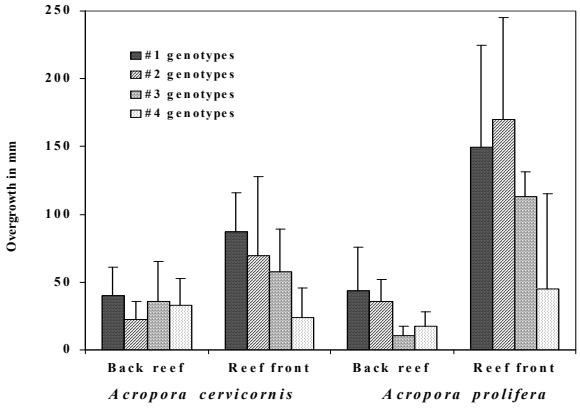

Figure 5: Mean overgrowth of *Acropora* genotypes in mm +SD onto wire mesh frames at one year (n= 15: 3 branches per genotype x 5 sites, 3 sites for reef front *A. prolifera*).

The experiment started with 10-cm fragments with little difference in thickness between apical fragments from the back reef and reef front. However, *A. cervicornis* from the reef front developed a distinctly more robust morphology than the back reef morphotype in the calm growth conditions of the back reef over the year. Statistically significant differences between the mean diameters of the two *A. cervicornis* morphotypes from the apical ($p=0.002$), middle ($p<0.00005$), and basal ($p<0.000005$) colony regions were revealed (Fig. 6) with *t*-tests. In addition to branch thickness, branch tips of the reef front morphotype were also noticeably softer and easily crushed, indicating a more porous, less heavily calcified skeleton than the lagoonal morphotype. Color differences were often apparent between the two morphotypes as well, the back reef type being more uniformly golden brown, while the robust reef front morph often had a darker brown or gray to purplish hue.

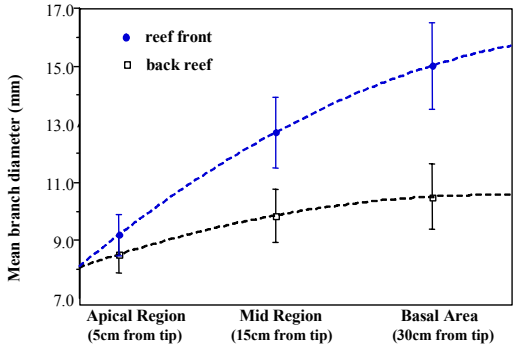

Figure 6: Comparison between reef front and back reef morphotypes of *Acropora cervicornis* grown together for one year, showing mean branch diameters ± SD for three colony regions.

Discussion

Based on this study, coral genotype appears to be more important than transplantation location, with 75% of all tests being significant for genotypic effects, as compared to only 44% of experimental outcomes having significant location effects. A G-test comparison of the observed frequencies confirms this ($p<0.005$, $G=8.3$, 1d.f.). However, genotype and location were equally as important for back reef morphotypes transplanted to the back reef, but for reef front morphotypes genotype was significantly more important than site in predicting experimental outcomes ($p<0.00001$, $G= 36.7$, 1d.f.). A potential explanation for the greater number of statistically significant differences among reef front corals transplanted to the back reef is that only certain genotypes from the reef front can adapt well to back reef environments, while genotypes from the back reef are in general well-adapted to the environment and therefore more even in their responses.

These results confirm that there is a biological basis for obtaining transplants from areas as similar as possible to the planned restoration site. *A. cervicornis* appears to be a highly diverse generalist species, not because each genotype has a high environmental tolerance, but because the species is composed of a diversity of genotypes, each adapted to a specific range of tolerances. Sourcing corals from environments similar to a restoration site and incorporating a high level of genetic diversity into transplantation projects would help ensure successful results for at least some of the transplants.

Based on genotypic differences alone, mean relative growth rates within *A. cervicornis* morphotypes growing together in the back reef varied by more than a factor of three, while mean relative growth rates within *A. prolifera* varied by a factor of six. These findings are in agreement with earlier studies which suggested that coral genotype strongly affected the ability of individual corals to thrive in a particular environment (Potts 1984; Edmunds 1994; Takabayashi and Hoegh-Guldberg 1995; Hoegh-Guldberg et al. 1997). A next step in advancing future research on the issue of environmental adaptation of coral genotypes might involve taking replicate fragments of specific genotypes and transplanting them into highly variable conditions of water flow, depth/light, and temperature.

A possible cause for the significantly slower growth and higher mortality of the reef front *A. cervicornis* genotype Fc4 could be that this genotype contains a maladapted zooxanthellae strain, perhaps even the zooxanthellae species identified by Baker et al. (1997), adapted to light conditions at greater than 9-12 m deep.

A possible non-genetic or non-zooxanthellae driven basis for differences in coral growth among genotypes could be the presence or absence of sublethal levels of pathogens within a corals sourced from the same thicket. In this study, there was an indication that disease may have suppressed the growth of genotype Fc4, which had significantly higher mortality ($p<0.0005$) than other genotypes. Of the 30 replicate Fc4 fragments, 66.7% died during the year, and much of this was visibly due to disease. This contrasts strikingly with the fastest growing genotype of this morphotype (Fc1), with only 3.3% mortality. Colonies of Fc4 appeared to be healthy during the first six months of the study, although displaying a noticeably slower growth rate. “White band” disease was later observed killing this genotype at several locations, while very few corals of the other genotypes had the disease. It appears that genotype Fc4 is either more susceptible to this disease, and the disease organisms were widespread throughout the reef system, or perhaps the disease was present in the

tissues of the source colony. The slower growth could have been the outcome of fighting a disease over several months, until the fragments eventually succumbed.

Bottjer (1980) studied the morphology of *A. cervicornis* from both reef front and back reef populations and surmised that the distinctly stockier morphology of the fore reef corals was environmentally induced. However this study suggests a genetic basis to this morphology. The reef front morphotype also had 14.6% less partially dead area in the lower portions of colonies than the back reef type (p=0.005, paired *t*-test), indicating that this morphotype of *A. cervicornis* invests more energy in maintaining healthy tissues in lower colony portions. The greater ability of reef front genotypes to attach themselves firmly to the substratum appears to be a genetically determined adaptation for survival and persistence in rocky reef front conditions, enabling coral fragments to attain a rapid and strong foothold. Baker et al. (1997) suggest the possibility of a cryptic species occurring within *A. cervicornis*, based on the occurrence of two distinctly different zooxanthellae taxa that were never found to co-occur in an individual coral. While the cryptic species proposed by them were segregated by depth, the *A. cervicornis* morphotypes of this study are segregated according to energy regime.

This study showed that morphological variation between back reef and reef front populations of *A. cervicornis* was relatively fixed. On the other hand, *A. prolifera* from the reef front changed growth form completely when grown in the back reef, transforming to become indistinguishable from the back reef genotypes. The thick-branched, open form described in Vaughan (1901), transformed into that of the undescribed slender-branched, bushy back reef type, indicating a plastic morphology based primarily on environmental influence. The back reef and reef front growth forms of *A. prolifera* thus conform to the definition of "ecomorphs", while the two forms of *A. cervicornis*, being independent of environmental causation are more accurately referred to as morphotypes.

If environmental conditions in the Caribbean have become unfavorable to the long-term survival of *A. cervicornis* as a species, coral transplantation might be used as part of a mitigation and recovery strategy. Artificially increasing the genetic diversity within monoclonal populations would potentially enhance natural fertilization rates, increasing sexual recruitment and genetic recombination and thus accelerating the recovery and adaptive potential of this ecologically important coral species. Another measure might include rescuing genetic material from declining *A. cervicornis* populations, transplanting and culturing coral fragments on frames as described in this study, as long as these transplantation efforts did not cause additional mortality. Reestablishing a more natural balance of herbivores on reefs where other factors such as siltation are not overpowering would potentially both increase the recruitment of coral larvae and the survival of remaining *A. cervicornis* populations. Effective interventions based on transplantation to conserve and restore *A. cervicornis* and *A. prolifera* will require further clarification of the population genetics of these species. Of course any recovery strategy will prove futile if the factors leading to coral reef decline in the Caribbean region are not reversed.

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Improving restoration approaches for *Acropora palmata*: Lessons from the Fortuna Reefer grounding in Puerto Rico

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Abstract. Detached *Acropora palmata* fragments (n=1857) generated by the *M/V Fortuna Reefer* grounding off Mona Island, Puerto Rico were secured to reef substrates or dead standing *A. palmata* skeletons using stainless steel wire. After 10 years, only 6% (n=104) of the fragments were alive, of which half (n=54) exhibited extensive branching (mean = 5 branches, 89 cm length), and a substantial increase in height (mean = 39 cm). Most surviving fragments were 20-100 cm (original length when first restored), secured to the reef and oriented upright. Fragments died or were lost in the first three years from wire breakage (23%), overgrowth by bioeroding clionid sponges (16%) and other factors (5%). Over the next 7 years, another 50% died due to gastropod predation, additional wire breakage and detachment during storms, and continued overgrowth by *Cliona*. Fragments attached to *A. palmata* skeletons initially grew rapidly and produced new branches, but most were subsequently dislodged due to bioerosion and breakage of the skeleton to which they were attached. Low fragment survival is attributed to 1) wire failure and inability of corals to overgrow wire, 2) limited fragment fusion, 3) attachment of fragments to inappropriate substrates, and 4) progressive mortality of large, older basal portions of detached colonies that failed to grow. Natural stressors unrelated to the restoration including diseases, predators, and bioeroding sponges have increased over time within and outside the grounding site and are also impacting fragment survival. Restoration may be a viable means to promote recovery of acroporid populations if the use of uncoated wire to attach fragments is avoided; fragments are placed on suitable substrates with live tissue in direct contact with the substrate to promote fusion, and efforts to mitigate disease and corallivory are undertaken.

Key words: *Acropora palmata*, Elkhorn coral, Restoration, Ship grounding, Coral disease.

Introduction

Since the listing of *Acropora palmata* on the U.S. Endangered Species Act in 2006, interest in recovering degraded and damaged populations of this species through restoration is increasing. Historically, most restoration efforts in the U.S. were conducted in response to ship groundings. Small scale restoration projects for *A. palmata* are now also being implemented after hurricanes and other catastrophic disturbances to minimize tumbling, scouring and loss of fragments associated with high wave energy and sand and rubble movement. Cement, epoxy, metal wire, cable ties, and/or molly bolts have all been used to reattach *A. palmata* fragments, with varying degrees of success (Precht 2006). Fragments have also been secured to artificial (cement) and natural (limestone rocks) structures, including reef crowns, rosettes, and limestone boulders, with further stabilization using molly bolts or other anchoring systems (Bruckner 2003). Fragments at the Fortuna Reefer site were attached to dead standing *A. palmata* skeletons to raise them off the substrate and to promote resheeting over dead skeletons (NOAA 1997; Iliff et al. 1999; Bruckner and Bruckner 2001).

The first major restoration effort for *A. palmata* in Puerto Rico was undertaken following the *M/V Fortuna Reefer* grounding on July 24, 1997. The grounding and removal of the vessel damaged shallow reef habitat dominated by *A. palmata* and *Diploria strigosa*. The reef substrate was crushed and fractured along the inbound and outbound tracks of the vessel, extending from the reef crest approximately 300 m seaward (2-4 m depth) and up to 30 m in width, with collateral damage to surrounding areas from cables used to extract the vessel (NOAA 1997).

An emergency restoration was completed within three months of the incident. The restoration focused on the reattachment of broken and dislodged *A. palmata* colonies and branches throughout the site of impact using stainless steel wire. Because of reports of frequent wire breakage and fragment loss, a mid-course correction using a stronger wire was undertaken in 2000 to prevent further fragment loss

(Bruckner and Bruckner 2001). This paper describes the fates of the fragments over 10 years, including patterns of survival, regrowth, and sources of mortality. The restoration techniques used at this site are evaluated and recommendations are presented on alternative approaches that may enhance the success of future restoration efforts for this species. Natural causes of mortality, unrelated to the grounding, are also discussed, along with options to mitigate these.

Material and Methods

Between September and October 1997, restoration experts stabilized detached *A. palmata* colonies and branches (n=1857) throughout the grounding site (2-6 m depth) off the southeast coast of Mona Island (18°02'N; 67°51'W; Fig. 1). Coral fragments (15-340 cm in length) were secured to the reef by wrapping stainless steel wire over coral fragments and around stainless steel nails that were inserted into pre-drilled pilot holes. Additional fragments were attached to dead standing *A. palmata* skeletons using wire and/or cable ties (NOAA 1997).

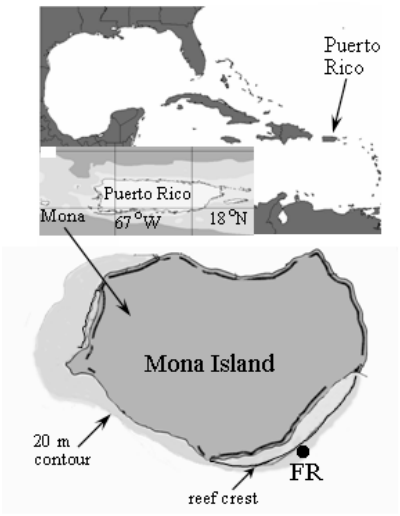

Figure 1: Location of the Fortuna Reefer (FR) restoration site.

We first examined the site in February 1998, and subsequently monitored changes to fragments and unmanipulated colonies and fragments in surrounding areas 1-3 times per year through February 2008. The initial survey involved an assessment of the number, size, and condition of fragments that remained attached, and the number of fragments that were detached and displaced or missing. Detached and missing fragments were identified from 1) groupings of nails within the reef without attached fragments; 2) remnant wire on skeletons that was not associated with fragments; and 3) unsecured fragments with remnants of attached wire that accumulated in sand channels or were scattered throughout adjacent fore and back reef habitats.

For each remaining fragment, measurements of the size (maximum length to nearest cm), orientation (up, down or sideways with respect to their orientation prior to breakage), origin (branch end, middle, or base of colony), location of attachment (reef or skeleton), and condition (live or dead) were recorded. Live fragments were also evaluated for tissue growth over the wire, presence and size of proto-branches, natural cementation (fusion) to the substrate, and resheeting over *A. palmata* skeleton. Estimates of size (length, width, height), percent remaining tissue, and old vs. recent mortality were made from a planar perspective using a 1 m bar (marked in 1 cm increments) oriented along the long axis of the fragment. Partial mortality was recorded as the percent loss from the upper surface of the reattached branches, and does not include the undersides of branches. All fragments were presumed to have 100% of their upper surface covered with tissue when first reattached in 1997.

Causes of partial or total mortality were identified as disease [white band disease (WBD), Caribbean ciliate infections, white patch (pox) disease, or growth anomalies], overgrowth by boring sponges (*Cliona* spp.), predation by snails (*Coralliophila abbreviata)*, polychaete worms (*Hermodice carunculata),* parrotfish *(Sparisoma viride*), macroalgal competition, or three-spot damselfish *(Stegastes planifrons*) territories. If the cause of mortality could not be determined, it was recorded as unknown.

Results

A total of 1857 fragments were reattached in 1997. Most surviving fragments (n=104; 5.6%) identified in February 2008 were attached to the reef (n=78; 75%) or dead *A. palmata* skeletons (n=26; 25%) and oriented upright (92%). A number of additional fragments that were originally attached to dead skeletons (n=28) had become detached due to wire failure and breakage of the underlying branches, and had accumulated in sand channels (Fig. 2). Over half (63%) of the surviving fragments had living tissue on most of their upper branch surfaces (>80%) and numerous branches (3-23 branches, 15-70 cm length); these had increased in height (20-80 cm) and width (15-150 cm). Other surviving fragments (37%, n=38) consisted of large (>80 cm length) middle and basal portions of broken colonies. These exhibited minimal signs of new growth over 10 years and a lack of fusion as the fragments were progressively losing tissue (mean=37% live tissue). Few fragments firmly fused to the reef substrate or coral skeletons (12%), and none of the fragments attached to dead skeletons successfully grew tissue over the underlying skeletal surfaces to which they were attached.

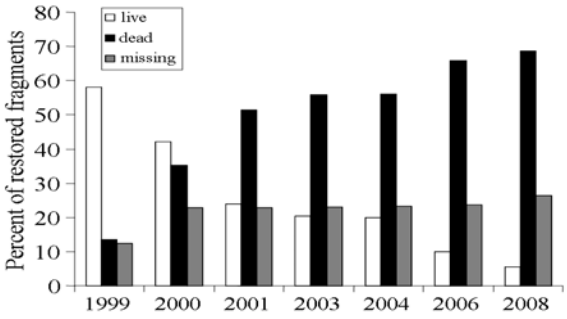

Figure 2: Changes over 10 years in the proportion of restored fragments (total=1857) that were still alive (white bars), dead (black bars) and detached and/or missing (grey bars).

Original fragment length, orientation (up or down), position on the source colony (e.g., branch end or middle) attachment location, and depth were important factors in the long-term survival of restored fragments (Fig. 3). The highest survival was recorded from 1.5-4 m depth, especially medium sized fragments (20-100 cm length) that were oriented upright and were attached to reef substrates. Small and medium-sized fragments attached to dead skeletons initially exhibited high rates of growth, but most were directed upward by production of new branches with only limited resheeting onto the dead coral substrates.

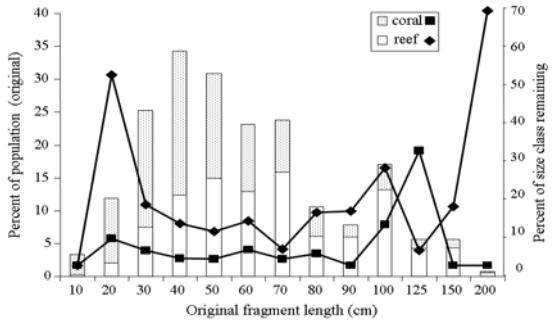

Figure 3: Percent of live fragments in each size class when initially restored (stacked bars) divided into fragments attached to the reef (white bars) and dead skeletons (grey bars), and the percent within each size class that was still alive after 10 years (line graph), attached to the reef (diamond) or to dead *A. palmata* skeleton (square).

Wire failure resulted in the loss of 23% (n=427) of the restored fragments in the first three years (prior to the mid-course correction). An additional 7% (n=55) of the fragments that were rewired in 2000 were lost during years 8-10, primarily due to breakage of underlying *A. palmata* skeletons as the fragments increased in size and branching complexity. Overgrowth by the boring sponge (*Cliona* spp.) was responsible for 22% total loss with 5% of the survivors currently affected by *Cliona* (Fig. 4). Mortality from *Cliona* was attributed to placement of fragments on or near substrates colonized by this sponge. Coral diseases identified throughout the site included WBD (7.3% of all fragments affected over 10 years), ciliate infections (3.2%), white patch disease (0.3%), and growth anomalies (1.3%). Numerous fragments died from WBD (5.8%), and the prevalence of WBD steadily increased from 2003-2008 (Fig. 4), while other diseases caused minimal partial mortality. Corallivorous gastropods (*C. abbreviata*) increased in abundance (maximum = 286 snails/survey) and density over time, affecting 17% (n=315) of all fragments over 10 years. These gastropods were found on fragments and colonies with WBD, as well as healthy corals, but they formed larger aggregations on stressed corals (6-40 snails per coral vs. 1-5 on "healthy" fragments), especially fragments that were dislodged and deposited in the sand. Damselfish algal lawns (1.1%), parrotfish predation (0.1%), and algal overgrowth (0.2%) were rare. The site was invaded by a crustose coralline algae (*Peyssonnelia* spp.) in 2006, which is rapidly spreading and has recently begun overgrowing corals. No bleaching was observed among *A. palmata* colonies or fragments during the 1998 and 2005 mass bleaching events or other years.

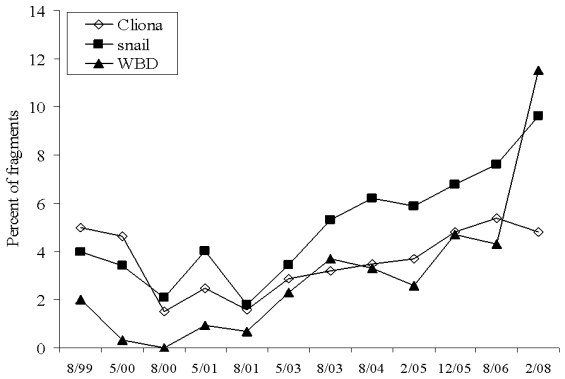

Figure 4: Major causes of partial and whole fragment mortality. A total of 22% of all fragments were overgrown by *Cliona* spp. (open diamonds), 5.8% were killed by WBD (triangles), and 17% were preyed on by *C. abbreviata* gastropods (square).

Discussion

Ten years after completion of the *M/V Fortuna Reefer* restoration, only about 6% of the fragments remain alive. About half of these have continued to grow and have produced numerous branches, recreating the structural complexity of an *A. palmata* reef. Other survivors, primarily the largest basal and middle portions of the colonies, failed to produce protobranches and are continuing to lose living tissue. A significant proportion of the fragments died from natural stressors unlikely to have resulted from the grounding or restoration. This includes pervasive and increasing predation pressure by *C. abbreviata* and an

outbreak of WBD that began five years after the grounding. Substantial loss in the first 2 years and over the longer term was also directly related to the restoration techniques used to secure the fragments, substrates selected for attachment, orientation of fragments, and attachment of larger, older basal parts of the colony with a lower likelihood of survival.

Attachment methods

"Traditional" methods to reattach coral fragments, including cement, epoxy, and cable ties were first attempted at the *M/V Fortuna Reefer* site. Cement offered the most efficient, natural, and cost-effective approach to secure fragments, but it was difficult to use due to the long setting time required and the heavy surge at the time of the restoration. A quick setting 2-part epoxy was also impractical because it was neutrally buoyant and larger fragments failed to properly adhere to substrates if algae, sediment, and sessile invertebrates were not thoroughly removed. Fragments secured initially to *A. palmata* skeletons with plastic cable ties were reported to loosen under surge conditions; thus, stainless steel wire was used (NOAA 1997; Iliff et al. 1999).

Over 10 years, wire proved to have numerous drawbacks. Considerable time was spent inserting stainless steel nails into reef substrates to provide a holdfast for the wire. This necessitated use of a hydraulic drill and a compressor to drill holes and cement to secure the nails. Another limitation of wire was the difficulty in securely attaching fragments to the reef to dampen wave surge effects. The slight, continuous movement of the fragments hampered their ability to fuse naturally and wire abraded the coral tissue, resulting in extensive partial fragment mortality (Bruckner and Bruckner 2001). Stainless steel wire used at the site also became pitted and broke, necessitating a mid-course correction after three years. Fragments were rewired using a stronger Monel nickel/copper alloy wire, but this also began to break after 4-5 years contributing to further fragment loss. Both types of wire also provided a substrate for accumulation of macroalgae and attachment of *Millepora alcicornis*, further compromising fragment health (Bruckner and Bruckner 2006b).

Cable ties are the optimal choice for reattachment of *A. palmata* on shallow exposed reefs, as coral tissue and skeleton is rapidly accreted over the plastic, and cable ties can be firmly tightened, although multiple ties may be required to prevent loosening. As a pilot experiment, we secured fragments removed from colonies with WBD to *A. palmata* skeletons using cable ties (n=23) and underwater two part epoxy. After four years only a single branch attached with cable ties was detached while all fragments attached with epoxy were lost; all other fragments secured with cable ties (94%) fused to the skeletons and overgrew the plastic (Bruckner and Borneman unpub. data). During the mid-course correction, a small number of fragments in shallow water were reattached to the reef substrate with cement. While several of these have died, none have become detached. Over the long-term, cement or some of the newer, commercial underwater epoxies like Allfix and Z-Spar may prove to be the most successful, cost-effective, and rapid method to reattach fragments, especially if the fragments are temporarily secured with plastic-coated wire or cable ties to allow time for cement to set. Plastic coated wire is likely to facilitate tissue overgrowth and reduce the likelihood of wire breakage but still must be monitored frequently to avoid loss of fragments due to storms.

Substrates for attachment

Fragments were attached to branches of dead standing *A. palmata* colonies with the expectation that they would attach to the dead branches and resheet over the skeleton, thereby restoring the three dimensional topographic relief much more rapidly (NOAA 1997; Iliff et al. 1999). Few fragments accreted tissue and skeletal material onto adjacent branches, and none grew back down the colony. Instead, most growth was directed upward in the form of new branches. These continued to increase in size and weight, creating more wave resistance until the underlying skeleton broke. The fragments were subsequently dispersed, overturned and deposited in sand flats, or removed from the site. Placement of fragments above the substrate had minimal benefits at reducing disease prevalence or corallivore abundance; and overgrowth by *Cliona* was actually higher than that observed in fragments attached to the reef (Bruckner and Bruckner 2006a), possibly because *Cliona* colonizes dead corals more frequently than the reef substrate. Fragments secured in an upright position to the base of colonies may have a better chance of fusion and resheeting as they grow upward.

Branch size and origin

Restored fragments ranged in length from 15-340 cm and included branch ends, as well as older colony bases and middle. Branch ends up to ~100 cm exhibited the highest rates of survival and growth, and greatest ability for natural fusion. Many of the largest basal and middle portions survived over 10 years, but they were affected to a greater degree by disease and corallivory, and very few developed new branches. In fact, most failed to grow at all, and instead slowly lost living tissue over 10 years. These fragments also often lacked tissue on their undersides when first restored, reducing their ability to fuse. Due to their large size, stabilizations and attachment is more labor

intensive, while small to medium sized branch ends can be relocated and secured more rapidly with cable ties, cement, or epoxy. Larger fragments could be positioned (but not attached) onto hard substrates to reduce scouring and smothering by sediment, and their weight and structural complexity alone may be sufficient to minimize movement. The time required to secure these could be better invested into attaching a greater number of smaller branch ends.

Enhancing natural fusion

Only a limited number of fragments, primarily smaller branch ends with live tissue on the upper surfaces and undersides when first attached, accreted tissue and skeleton onto the underlying substrate over 10 years. Other branches were unable to reattach, mainly because the branches were not positioned such that living tissue was directly in contact with the substrate, and fragments exhibited slight back and forth movement during wave surge. Fusion may be enhanced by placing fragments at a slight angle such that a portion of the living surface of the branch was in contact with exposed, algal-free substrate. Ensuring contact of living tissue with a hard substrate free of algae may provide adequate time for natural fusion, thereby minimizing losses at a later stage when the wire breaks.

Controlling natural stressors

The gastropod *Coralliophila abbreviata* is a significant predator of *A. palmata* and may also be a vector for disease (Bruckner et al. 1997; Williams and Miller 2005). If snails occur within the restoration site, these should be removed while the restoration is underway, with additional removal as needed during annual monitoring to minimize tissue loss. Collection of *C. abbreviata* into sealable plastic bags for disposal on land is recommended due to their brooding life history strategy. We conducted a snail removal experiment from this site in Aug 2006. After 1.5 years, a large number of snails recolonized the area, but far fewer than before (Bruckner and Borneman unpubl. data). Although the causative agent of WBD remains elusive, and few attempts to treat WBD-affected corals have been undertaken, branch ends removed from colonies with WBD and attached to surrounding substrates exhibited high rates of survival, while the source colonies completely died (Bruckner and Borneman unpubl. data). One other stressor, the bioeroding sponge *Cliona*, readily overgrew fragments placed on or next to it. *Cliona* also spread to new areas, increasing in cover over the duration of this study. Placement of fragments away from competitive species (such as, *Cliona* and *Palythoa*), minimizes the likelihood of overgrowth, and may allow ample time for fusion and growth of fragments.

Success of future restoration efforts directed at this species may be enhanced by taking into consideration the environmental conditions affecting the site and suitability of the site for restoration including physical factors such as wave exposure, condition of reef substrates (e.g., cover of macroalgae and bioeroding sponges), and presence of disease and corallivores. Specific restoration techniques including the use of materials that do not negatively affect living tissue, placement of fragments on suitable (cleaned) substrates, orienting fragments such that live tissue contacts the substrate, and selection of small to medium sized branch ends can enhance survival and possibly speed up ecological recovery of the site.

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The effectiveness of macroalgal reduction and *Diadema antillarum* addition in limiting macroalgal growth and facilitating coral recovery

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Abstract. The effectiveness of macroalgal reduction and *Diadema antillarum* addition in limiting macroalgal growth and facilitating coral recovery was investigated between June 2002 and July 2003. Two treatments, one involving the manual reduction of macroalgae, and another involving macroalgal reduction followed by the addition of *D. antillarum*, were each performed on a set of three replicate patch reefs off the island of Eleuthera, The Bahamas. Mean macroalgal cover was reduced to <2% immediately after macroalgal reduction, but neither treatment was effective at preventing macroalgal re-growth after 12 months. Mean juvenile coral density increased by more than 150% immediately after macroalgal reduction. No significant change in mean coral cover was detected for either treatment or the control between 0 mo and 12 mo and an increase in mean juvenile coral density observed on some reefs during this period cannot be attributed to the effectiveness of either treatment. The failure of the treatments in significantly limiting macroalgal growth and facilitating coral recovery was likely due to poor *D. antillarum* survivorship. The results suggest that the effectiveness of reef restoration efforts involving translocated or lab-reared *D. antillarum* may be limited on some reefs and may require multiple re-stocking efforts.

Keywords: Reef restoration, Macroalgal removal, *Diadema antillarum*, Bahamas, Patch reefs

Introduction

The vitality of coral reefs worldwide continues to decline despite heightened concern and the substantial efforts committed by hundreds of government agencies and nongovernmental organizations (Birkeland 2004). It may be necessary to supplement efforts to address climate change, overharvesting, coastal eutrophication, sedimentation and other threats with direct intervention at a local scale to help spur reef recovery in areas where these threats have been minimized, but where reef recovery has not been evident. The aim of this investigation was to assess the combined effectiveness of macroalgal reduction and the addition of *Diadema antillarum* Philippi on patch reef communities in the Bahamas. Macroalgal reduction, combined with the enhancement of *D. antillarum* population densities, may help facilitate localized phase-shift reversal on Caribbean/Western Atlantic coral reefs.

Materials and Methods

This study was conducted between June 2002 and July 2003 on a series of small patch reefs (4.9-8.3 m^2) found in 10 to 11 m of water off the northwestern coast of Cape Eleuthera, Eleuthera, The Bahamas. Nine experimental reefs were selected at random from a set of 20 patch reefs. Separate sets (= three replicates) of reefs received one of two treatments: macroalgal reduction (Treatment A) or macroalgal reduction combined with the addition of *D. antillarum* individuals (Treatment B). A third set of reefs did not receive any treatment and served as the control. Macroalgae were removed from the experimental reefs by hand. *Diadema antillarum* were collected from a nearby fringing reef and translocated to Treatment B reefs. The urchins were individually placed on the reefs with a target population density of approximately 4 m^{-2}. All Treatment B reefs were re-stocked with urchins at t = 5 mo, as no urchins survived from the initial translocation.

Surveys designed to monitor changes in coral and macroalgal cover, algae biomass, juvenile coral density, and coral health were conducted on the experimental reefs during the course of the 12-month investigation. Macroalgal and coral percent cover was quantified by filming non-overlapping transects placed across the length of each reef with a digital video camera at t = 0 (pre-treatment), t = 0^{post} (immediately post-treatment), t = 5, and t = 12 mo. The number (2-3) and length (2-4 m) of the transects varied depending on the size of reef. Percent cover estimates were obtained by analyzing consecutive, non-overlapping video frames using PointCount99®. Twenty-five randomly-generated points were used for

each frame; a minimum of thirty frames were analyzed for each reef. The percent cover value for each video frame was considered a sample; all samples from a single reef were pooled to obtain mean macroalgal and coral cover values for each reef. For comparison between treatments, mean macroalgae and coral cover values for each treatment were obtained by pooling all samples from all replicate reefs.

Algae were collected from four random sample quadrats (0.0625 m^2) for each reef at t = 0 and t = 12 mo. All macroalgae and most turf algae were removed from each quadrat and placed into individual sample bags. The samples were rinsed and separated into algal groups: fleshy green, fleshy brown, and calcareous green. The samples were dried and then weighed using an Acculab v1-200 balance (200g capacity, 0.01 g readability). The mean algae dry-weight value for each reef was obtained by averaging the algae dry-weight values for all samples taken from each reef. Mean algae dry-weight values for each treatment were obtained by pooling all samples for all reefs that received the same treatment.

Attempts to conduct a visual census of all juvenile corals on Treatment A and Treatment B reefs were made at t = 0 mo, t = 0^{post} mo, and at t = 12 mo, while the control reefs were surveyed only at t = 0 mo and at t = 12 mo. Juvenile corals were located by closely inspecting the substratum; they were identified to species when possible. Measurements (mm) were taken at the longest axis of the polyp or colony. Only corals with 20 polyps or fewer and a diameter $\leq$ 4cm were counted. Mean juvenile coral count values for treatments were obtained by averaging the total counts for reefs receiving the same treatment at each sampling period. The total number of juvenile coral individuals was normalized by reef area and reported as density (colonies m^{-2}). A visual census for adult and juvenile *D. antillarum* was conducted in conjunction with the juvenile coral survey.

Three 0.0625 m^2 permanent quadrats were established on each reef prior to treatment and filmed at t = 0 mo, t = 0^{post} mo, t = 5 mo, and at t = 12 mo, to examine fine-scale changes in the benthic community, focusing particularly on coral/algal interactions and sources of coral mortality and tissue stress.

An equal variance *t*-test was used to compare pooled treatment data between various time intervals. The equivalent non-parametric test (Mann-Whitney *U* test) was used when data did not satisfy the assumptions of normality and homoscedasticity or if the sample size was very small (n < 5). A one-way ANOVA or the equivalent non-parametric test (Kruskal-Wallis one-way ANOVA on ranks) was used to compare treatments and reefs at each time period; Tukey-Kramer Multiple Comparison test or the equivalent non-parametric test (Kruskal-Wallis Multiple-Comparison Z-value Test) was used to determine the sources of significance.

Results

Macroalgal reduction lowered mean macroalgal cover to approximately 2% on both Treatment A and Treatment B reefs between t = 0 and t = 0^{post} mo (Mann-Whitney *U*, $p < 0.001$)(Fig. 1A). Treatment A reefs and the control reefs exhibited increases in mean macroalgal cover of 31.4% and 19.3%, respectively, between t = 0 and t = 12 mo (Mann-Whitney *U*, $p < 0.001$; equal variance *t*-test, $p < 0.001$). No change in mean macroalgal cover was detected on Treatment B reefs between t = 0 and t = 12 mo. Mean scleractinian coral cover on Treatment A and Treatment B reefs appeared to increase by 66.7% and 50% respectively, immediately following treatment, but these change were not significant (Mann-Whitney *U*, $p = 0.078$ and 0.091, respectively). No significant changes in mean scleractinian coral cover were observed for either treatment or the control between any other sampling periods (Fig. 1B).

In contrast to the decrease in algae biomass expected for Treatment A and Treatment B reefs, both treatments and the control exhibited a significant increase in mean algae dry weight after 12 mo (Fig. 1C). Mean dry weight values on Treatment B reefs had increased the most (72.3%), while increases of 41.6% and 60.4% were observed on Treatment A and the control reefs, respectively (Mann-Whitney *U*, $p = 0.001$, 0.002, and 0.009, respectively). Fleshy, green macroalgae dominated the algae biomass samples, with a mean percent composition across all samples of 93.1 ± 9.4%. Visual estimates suggest that *Microdictyon marinum* comprised the majority of the dry-weight for all the samples.

Mean juvenile coral density increased 286% on Treatment B reefs immediately after treatment (Mann-Whitney *U*, $p = 0.05$) (Fig. 1D). Treatment A reefs exhibited a 165% increase in mean juvenile coral density during this period, but the change was not significant at the 0.05 level (Mann-Whitney *U*, $p = 0.092$). Mean juvenile coral density on Treatment A reefs decreased 49.3% between t = 0^{post} and t = 12 mo (Mann-Whitney *U*, $p = 0.05$). Mean juvenile coral density on Treatment B reefs appeared to decrease by 55% during this period, but the change was not significant (Mann-Whitney *U*, $p = 0.1$) Mean juvenile coral density had increased by 95.7% on Treatment B reefs between t = 0 and t = 12 mo (Mann-Whitney *U*, $p = 0.038$). An apparent increase of 174% was observed on the control reefs during this period, but the change was not significant (Mann-Whitney *U*, $p = 0.1$). No significant increase was

seen on Treatment A reefs between t = 0 and t = 12 mo (Mann-Whitney *U*, p = 0.329).

No adult urchins were found on any of the experimental reefs at t = 0, t = 5, and t = 12 mo. No juvenile *D. antillarum* were found on any of the reefs prior to treatment; one juvenile was found on one reef undergoing Treatment A, immediately after macroalgal removal. Three juveniles, one on each of two reefs undergoing Treatment B and one reef undergoing Treatment A, were found at t = 5 mo, while none were found on any reefs at t = 12 mo.

Examination of the permanent quadrat video frames revealed evidence of the partial and full mortality of several coral colonies. Partial mortality and tissue discoloration as a result of macroalgal overgrowth or abrasion was evident on several colonies. White Plague appeared responsible for the full mortality of a colony of *Dichocoenia stokesii* on a Treatment A reef, and Red Band Type II was likely responsible for the partial mortality of a *Montastrea faveolata* colony on another Treatment A reef. Infestation by the boring sponge, *Cliona* sp., was also evident on several colonies.

Discussion

Mean macroalgal cover decreased markedly on both Treatment A and Treatment B reefs immediately after macroalgal reduction, but the low macroalgal cover and biomass did not persist on either set of reefs. Even though Treatment B appeared to be more effective than Treatment A in limiting an increase in macroalgal cover, Treatment B was not able to prohibit the re-growth of macroalgae. Even more striking than the increase in mean macroalgal cover exhibited by Treatments A and the control reefs between t = 0 mo and t = 12 mo was the increase in mean algae biomass exhibited by all treatments during this same time interval. This increase suggests that the treatments were ineffective at reducing algal biomass and even at preventing algal biomass from increasing during this period. The observation that algae biomass samples were dominated by fleshy green algae, and, in particular, by *Microdictyon marinum*, is in concordance with reports of extensive *M. marinum* blooms on several Bahamian islands (Woodley et al. 2000; Kramer et al. 2003; Peckol et al. 2003). Coral tissue stress and mortality caused by *M. marinum* has been observed elsewhere in the Bahamas (Dustan 1987; Kramer et al. 2003; Peckol et al. 2003). The likely increase in mean scleractinian coral cover on Treatment A and Treatment B reefs between t = 0 and t = 0^{post} can be attributed to the detection of live coral that had been hidden beneath the thick macroalgal canopy prior to macroalgal reduction.

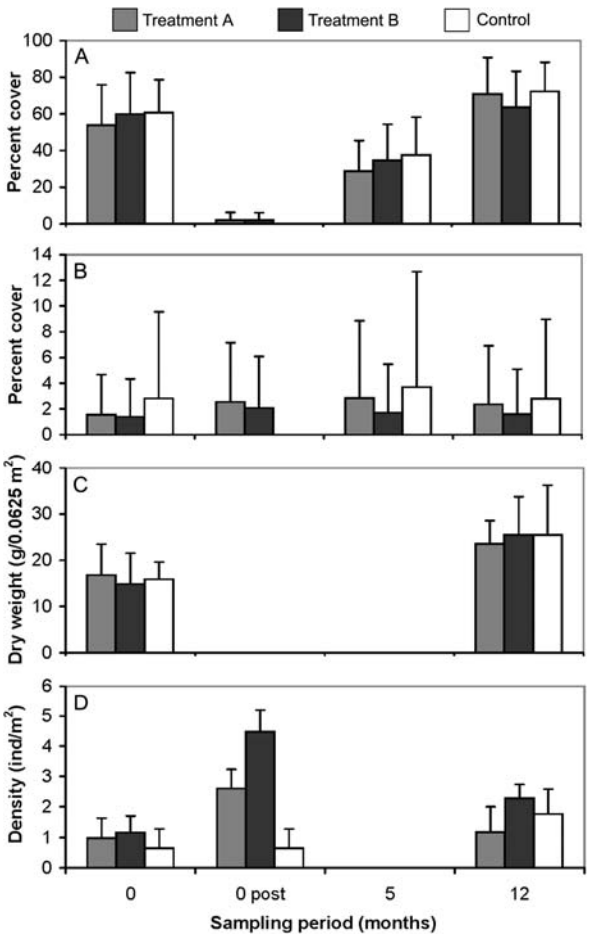

Figure 1: Mean macroalgal cover (A), mean scleractinian coral cover (B), mean algae dry weight (C), and mean juvenile coral density (D) at each sampling period. Algae dry weight data was not collected at t = 0^{post} and t = 5 mo; juvenile coral surveys were not conducted at t = 5 mo. Error bars represent + SD.

The significant increase in mean juvenile coral density on Treatment B reefs, and the likely increase observed on Treatment A reefs immediately after treatment indicates that the thick macroalgal canopy covered a substantial number (approximately two-thirds) of juvenile recruits. The presence of such a large number of juvenile corals beneath the algal canopy strengthens the case for macroalgal reduction, which would increase their access to sunlight and food – if only temporarily. Some corals evidently recruited successfully despite high macroalgal cover, but their growth and survival was likely limited (Miller and Hay 1996; Edmunds and Carpenter 2001). The absence of any significant increase in juvenile coral density on Treatment A reefs and the apparent increase on both Treatment B and the control reefs between t = 0 and t = 12 mo suggest that the treatments were not responsible for any increase in mean juvenile coral density and that natural variability is a more likely explanation.

The decline in coral cover and vitality in Treatment A quadrats observed during this investigation can be attributed to active tissue necrosis resulting from at least two coral diseases/disease states, and to a lesser degree to macroalgal-related stress (e.g., abrasion, shading, and sediment stress). The active tissue destruction of the few remaining medium-to-large coral colonies by White Plague and Red Band Disease, combined with macroalgal overgrowth-associated tissue mortality, suggests continued decline in the vitality of the experimental site coral community.

Examination of the permanent quadrat video frames as well as observations made throughout the study also provide evidence of potentially significant infestation of coral heads by the boring sponge, *Cliona* sp. If, in fact, the incidence and severity of clionid sponge infestation on the experimental reefs were anomalously high, it could have been an indication of excess nutrient input (Rose and Risk 1985; Ward-Paige et al. 2005) and/or the result of the opportunistic infestation of colonies weakened or freshly killed by bleaching, disease, or other sources of stress (Schonberg and Wilkinson 2001).

The effectiveness of the combined treatment of macroalgal reduction and *D. antillarum* addition was likely limited by poor *D. antillarum* survivorship and may have been obscured by the onset of environmental factors more favorable to increased macroalgal growth. Despite restocking the experimental reefs with urchins five months after the initial treatment, none remained at the termination of the experiment. Based on the limited distribution and foraging of *D. antillarum* observed in other studies as a result of high levels of predation pressure (Carpenter 1984; Levitan and Genovese 1989), the urchins were expected to remain on the experimental reefs. Even when food supply is limited, *D. antillarum* tend to avoid the risks associated with migration and instead have the ability to reduce test growth rates and test size in more severe food-limited situations (Levitan 1988).

The low *D. antillarum* survivorship rates observed in this study are similar to those observed in a recent study that involved enclosing aggregates of wild *D. antillarum* around individual coral colonies in an attempt to improve the substrate quality for coral reseeding efforts and supplementing local *D. antillarum* populations with lab-reared individuals and monitoring their survivorship (The Nature Conservancy 2004). *Diadema antillarum* mortality was high in both components of the study, with approximately two-thirds of the enclosed wild urchins lost within the first 3 to 6 months of the project and 100% of the lab-reared urchins lost between 24 hours and several weeks after their release. Predation was cited as the major source of the high mortality levels in both cases. Despite the low urchin survivorship observed in the Nature Conservancy (2004) study, high levels of algae biomass were removed by the wild urchins and coral spat were observed several months after collected coral spawn were seeded on the primed substrate.

In contrast to the low *D. antillarum* survivorship observed in this investigation and in the Nature Conservancy (2004) study, Nedimyer and Moe (2003) observed considerably greater survivorship (30% remained after 11 mo) in a similar effort that involved translocating juvenile *D. antillarum* from high settlement/high mortality reef crest rubble zones to deeper patch reefs. The significantly greater *D. antillarum* survivorship in the Nedimyer and Moe (2003) study was likely one of the major factors that contributed to the increased scleractinian coral cover, decreased macroalgal cover, and the increased juvenile coral density observed on their experimental reefs a year after the urchin additions.

The low *D. antillarum* survivorship observed in this study may have been controlled by several factors, such as the potential lack of suitable refugia, the presence of more robust populations of *D. antillarum* predators, or a combination of factors. The patch reefs used by Nedimyer and Moe (2003) were between 44 and 96 m^2, and may have provided more suitable urchin refugia.

In addition to the substantially greater *D. antillarum* survivorship observed in the Nedimyer and Moe (2003) study, the difference in benthic community compositions between the two sets of reefs prior to treatment may also have influenced the differing outcomes of *D. antillarum* additions of the Nedimyer and Moe (2003) study and this investigation. For example, scleractinian coral cover on the Nedimyer and Moe (2003) reefs was significantly higher (ranging from 5.5-14%, mean of 9.8%), while coral cover on the reefs examined in this investigation ranged from 0.7 to 5.6%, with a mean of 1.9%. Mean algae cover on the Nedimyer and Moe (2003) reefs was 54.3%, with well over half of the total algae cover on all reefs consisting of turf algae. Brown foliose algae cover ranged from 9-11%, while green foliose algae was less than 1% on all of the Nedimyer and Moe (2003) reefs. In contrast, macroalgal cover alone (not including turf algae) on the reefs in this study ranged from 37.2-71.0%, with a mean of 58.2%.

Also, *Microdictyon marinum* was not mentioned in the Nedimyer and Moe (2003) study. If it was present at all, it was only a minor component of the benthic community. This is in stark contrast to the dominance of *M. marinum* on the reefs of this study, which may have had a strong influence on the effect of the

experimental treatments. The rapid growth of *M. marinum* and the high canopy height achieved by the species may have a more profound affect on coral survivorship than other algae species. Its rapid growth, in combination with the poor survivorship of *D. antillarum*, may also have prevented the maintenance of an algal turf by overwhelming the urchins and the herbivorous fish. It is also interesting to note that although Nedimyer and Moe (2003) did not utilize macroalgal reduction to "prep" the substrate for the addition of *D. antillarum*, significant decreases in macroalgae and increases in coral cover were still observed on the experimental reefs.

Conclusions and Recommendations

The results of this study suggest that the proposed restoration technique in its present form was not effective at significantly reducing macroalgal re-growth or enhancing juvenile coral recruitment. The unexpectedly high flight and/or mortality rates for the translocated *D. antillarum* individuals are the most likely explanations for the less-than-expected performance of the restoration technique. One would expect a persistent *D. antillarum* population to be able to maintain an algal turf and prevent macroalgal re-growth, but the number of urchins needed to sustain such a population in the face of high flight/mortality rates would require a considerable supply of urchins and considerable human effort. Survivorship may be greater at other sites, as Nedimyer and Moe (2003) observed, and thus the success of the treatment may vary from site to site as a function of urchin predation levels, availability of suitable refugia, or some other factor.

Future restoration efforts using *D. antillarum* may benefit from abandoning patch reefs of such small sizes in favor of larger patch reefs – perhaps those with an area of 25 to 50 m^2. The larger size may enhance *D. antillarum* survivorship by providing more refugia. Macroalgal reduction on patch reefs of this size would still be feasible with a relatively small number of divers.

The development of a restoration technique involving macroalgal reduction and the addition of *D. antillarum* that was more successful at reducing macroalgae and increasing adult coral cover and juvenile coral density could be used concomitantly with efforts to manage local anthropogenic threats. Efforts to reduce anthropogenic impacts to coral reefs on a local level, and the implementation of localized restoration efforts should be continued and can be considered attempts at restoring to the reef-building corals the resilience required to acclimate or adapt to rapidly changing environmental conditions.

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Benthic colonization and ecological successional patterns on a planned nearshore artificial reef system in Broward County, SE Florida

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Abstract. Nearshore marginal reefs are frequently impacted from beach restoration activities. Beach nourishment can impact nearshore hardbottom through burial and subsequent habitat degradation. In response to increased beach nourishment activities, reef restoration projects have increased immensely over the last decade. In Southeast Florida, the deployment of artificial reefs (ARs) as mitigation for nearshore habitat loss has become routine. To determine the success of ARs as appropriate nearshore habitat replacement, long-term AR colonization studies are essential to evaluate ecological processes and succession rates in shallow marine environments. This paper reports on the development, recruitment and ecological succession rates of macrobenthic communities on a mitigative AR system located in Broward County, Florida. Pioneering organisms and community composition were evaluated via the Coastal Planning & Engineering, Inc. Benthic Ecological Assessment for Marginal Reefs (BEAMR) method. Benthic communities were sampled at 9-, 12-, 18-, 24-, and 36-month post-deployment. Both spatial and temporal fluctuations of colonizing organisms were assessed, as well successional stages of contemporary organisms. Results indicated a typical colonization pattern where biotic cover and diversity increased on the AR system progressively from 9 to 36 months post-deployment; however, equilibrium was not attained at the functional group level due to frequent physical disturbances.

Key words: Artificial reef, community development, recruitment, macrobenthic communities, functional group.

Introduction

Beach nourishment can impact nearshore hardbottom through burial and subsequent habitat degradation. In response to increased beach nourishment activities, reef restoration projects have increased immensely over the last decade. In southeast Florida, the deployment of artificial reefs (ARs) as mitigation for nearshore habitat loss has become commonplace. To determine the success of ARs as appropriate nearshore habitat replacement, long-term AR colonization studies are essential to evaluate ecological processes and succession rates in shallow marine environments (Cummings 1994; Perkol-Finkel and Benayahu 2005; Thanner et al. 2006).

Understanding the spatial and temporal fluctuations of colonizing organisms is essential in forecasting benthic community development on ARs. Classic models of colonization suggest a directional successional sequence where pioneering species rapidly colonize the substrata in high numbers followed by a more diverse climax community (Bailey-Brock 1989; Cummings 1984; Palmer-Zwahlen and Aseltine 1994; Stanos and Simon 1980). Alternatively, Sutherland and Karlson (1977) suggest that classic succession cannot be evaluated with a new AR system as recruitment is determined by stochastic larval recruitment and the relative dominance of pioneer species. Additionally, biological habitat complexity is an important factor pertaining to larval settlement and species composition; thus, only after a temporal community shift will additional species have the opportunity to settle onto suitable habitat (Connell and Slather 1977).

In 2003, approximately 9 acres (3.6 ha) of limestone boulders were deployed in 5-m water depths between Florida Department of Environmental Protection (FEDP) R-monuments 101 and 125. The diameter of each boulder was 1.2-1.8 m. The limestone boulders, acquired from a Grand Bahamian quarry, were placed in a single layer on hardbottom areas covered with approximately 0.9 m of sand. The distance between individual boulders did not exceed 2.1 m. This study reports on the biotic community development and ecological succession patterns on this AR system located in Broward County, Florida (Fig. 1).

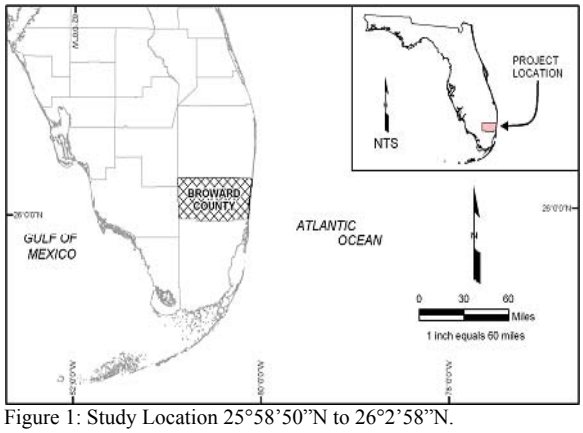


Figure 1: Study Location 25°58'50"N to 26°2'58"N.

Material and Methods

Benthic community composition was measured 9, 12, 18, 24 and 36 months after deployment using the Coastal Planning & Engineering, Inc. Benthic Ecological Assessment for Marginal Reefs (BEAMR) method (Lybolt and Baron 2006). A component of the BEAMR protocol includes a standardized BEAMR datasheet which prompts biologists to complete all fields *in situ* and strengthens quality assurance (Fig. 2).

Sample Name or # 2.5		List macroalgae Genus List every coral colony ~and coral condition(s)	% cover max size (cm)
Max Relief (cm)	4	Hypnea	1
Max Sediment Depth (cm)	1	Gracilaria	1
Sessile Benthos...	% Cover		
Sediment- (circle all (sand) shell mud)	20		
Macroalgae- Fleshy+Calcareous	3		
Turf- algae+cyanobacteria (circle all: (g) (r) b)	60		
Encrusting Red Algae	0	S. hyades	14
Sponge	2	S. siderea	5
Hydroid	0		
Octocoral	0		
Stony Coral	1		
Tunicate	0		
Bare Hard Substrate	14		
other-...	0		
Total Must = 100%			

Figure 2: Example of a completed BEAMR datasheet.

BEAMR samples three core characteristics in each quadrat: physical; abiotic and biotic percent cover; and coral density. Physical characteristics recorded are maximum relief in the quadrat (to nearest cm) and maximum sediment depth (to nearest cm). Visual estimates of planar percent cover of all sessile benthos are pooled to 19 major functional groups. Macroalgae percent cover data are reduced to genus-level percent cover (all genera with at least 1% cover). Each colony of octocoral and stony coral is identified and the maximum height or width is measured to the nearest cm. Octocoral individuals are identified to genus and stony coral individuals are identified to species whenever possible. The smallest size recorded is 1 cm, which includes individuals less than or equal to 1 cm. As with all non-consumptive surveys, BEAMR is necessarily constrained to visually conspicuous organisms with well defined discriminating characteristics for identification.

A total of 26 transects were sampled at each monitoring event. Prior to benthic data collection, stainless steel pins were installed on each transect at 0, 1, 5, 10, 15, 20, 25, 29, and 30 m to ensure permanent sampling sites. BEAMR was conducted on the north side of each 30-m transect using 12 replicate 1.0-m^2 quadrats (1 m x 1 m) every 2.5 m, starting at 0.0-m. The southwest corner of each BEAMR quadrat was aligned with the precise point of the sample location.

Non-parametric multivariate analyses were performed using PRIMER-E® (v6) (Clarke and Gorley 2006; Clarke and Warwick 2001). The biotic dataset was standardized by total and transformed by a log (X+1) transformation. A Bray-Curtis similarity matrix was also applied. Multi-dimensional scaling (MDS) ordinations and analysis of similarity (ANOSIM) routines were derived from the Bray-Curtis similarity matrix to determine if significant changes in the biotic assemblage occurred over time. Diversity indices were also comparatively analyzed.

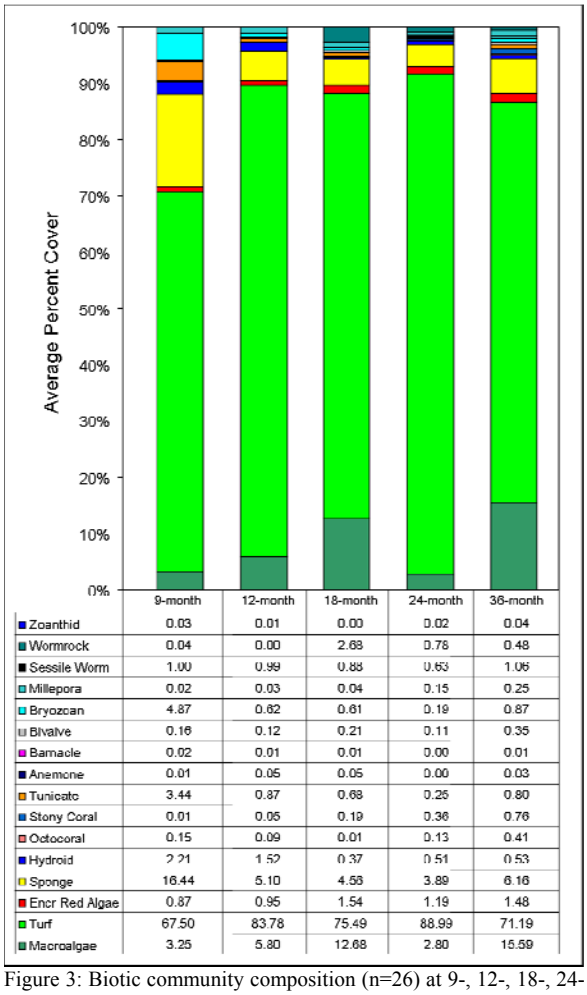
Results

The biotic benthic community of the artificial reef was analyzed in order to determine recruitment patterns. Fig. 3 presents the biotic community on the artificial reef at the five discrete monitoring times. Turf algae was the dominant taxon over time. Of the faunal functional groups, sponges (16.4%), bryozoans (4.9%), tunicates (3.4%), and hydroids (2.2%) were highest during 9-month post-deployment but decreased sharply thereafter.

Sponge average percent cover decreased after 9 months and remained at approximately 5% for the remaining monitoring events. Scleractinian corals and *Millepora* spp. demonstrated the most consistent increase in abundance over time (Fig. 3).

A similarity percentages (SIMPER) analysis identified discriminating functional groups between each monitoring survey. Between 9-month and all subsequent monitoring surveys, bryozoans and

macroalgae were the primary contributors to the dissimilarity (Fig. 4).



	9-month	12-month	18-month	24-month	36-month
Zoanthid	0.03	0.01	0.00	0.02	0.04
Wormrock	0.04	0.00	2.68	0.78	0.48
Sessile Worm	1.00	0.99	0.88	0.63	1.06
Millepora	0.02	0.03	0.04	0.15	0.25
Bryozoan	4.87	0.62	0.61	0.19	0.87
Bivalve	0.16	0.12	0.21	0.11	0.35
Barnacle	0.02	0.01	0.01	0.00	0.01
Anemone	0.01	0.05	0.05	0.00	0.03
Tunicate	3.44	0.87	0.68	0.25	0.80
Stony Coral	0.01	0.05	0.19	0.36	0.76
Octocoral	0.15	0.09	0.01	0.13	0.41
Hydroid	2.21	1.52	0.37	0.51	0.53
Sponge	16.44	5.10	4.58	3.89	6.16
Encr Red Algae	0.87	0.95	1.54	1.19	1.48
Turf	67.50	83.78	75.49	88.99	71.19
Macroalgae	3.25	5.80	12.68	2.80	15.59

Figure 3: Biotic community composition (n=26) at 9-, 12-, 18-, 24- and 36-month post-deployment.

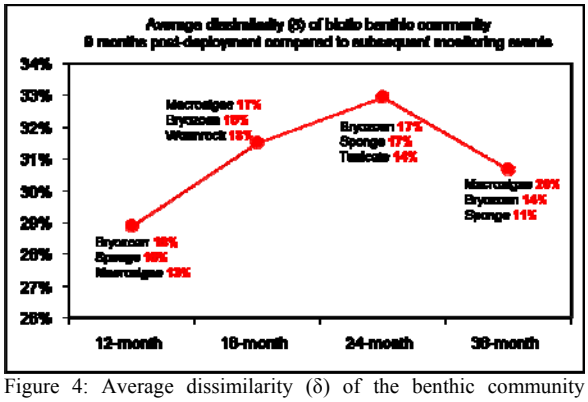


Figure 4: Average dissimilarity (δ) of the benthic community between the 9-month and subsequent monitoring events. Percentages of the major contributors to δ are listed at each event.

Initially bryozoan cover was relatively high at 4.9% but decreased rapidly 3 months later to less than 1% where it remained consistently thereafter. Macroalgae cover increased consistently until 24-month where percent cover decreased to less than 3% (refer to Fig. 3). However, macroalgae percent cover rebounded to almost 16% at the 36-month monitoring event. Stony coral became a dominant contributor (8.8%) to the community dissimilarity between 12 and 36 months.

Non-parametric multivariate analyses were applied to the functional group dataset to further examine changes in biotic composition over time. A MDS ordination with cluster overlay indicated a distinct difference between the biotic assemblage at 9-month post-deployment compared to subsequent monitoring intervals (Fig. 5). However, the functional group assemblages remained at least 75% similar throughout all monitoring surveys. Additionally, results of a 1-way ANOSIM revealed the 24-month post-deployment biotic community most closely resembled the 12-month community (R=0.273). This similarity correlates with the decrease in macroalgae cover from 12.7% at 18-month to 2.8% at 24-month (see Fig. 3). The 1-way ANOSIM also revealed the strongest biotic differences existed between 9- and 36-month assemblages (R=0.677), and between the 24- and 36-month assemblages (R=0.655).

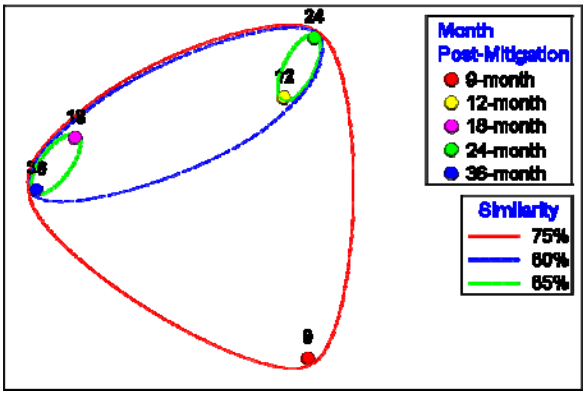


Figure 5: MDS ordination based on Bray-Curtis similarity of the biotic assemblages on the artificial reef at 9-, 12-, 18-, 24-, and 36-month post-deployment (stress = 0.1).

Diversity indices were calculated on the non-transformed data at each monitoring survey utilizing PRIMER (Table 1). The overall trend showed an increase in community diversity over time.

Table 1: Diversity indices of the biotic benthic community over time.

	No. of Functional Groups (S)	Margalef's (d)	Pielou's (J')	Shannon's H' (loge)	Simpson's (1-d)
9-month	16	5.93	0.69	1.92	0.86
12-month	15	5.87	0.68	1.85	0.85
18-month	16	6.93	0.72	1.99	0.88
24-month	14	5.62	0.71	1.87	0.84
36-month	16	5.72	0.77	2.12	0.90

The macroalgae community was analyzed to determine which genera were the initial colonizers and successive genera (Fig. 6). *Padina* spp. had the

highest percent cover at 9-month (3.7%) while *Wrangelia* spp. dominated the substrate at 36-month (76.7%). *Dasya* spp. was most prevalent at 18-month (55.2%), which was the only winter survey.

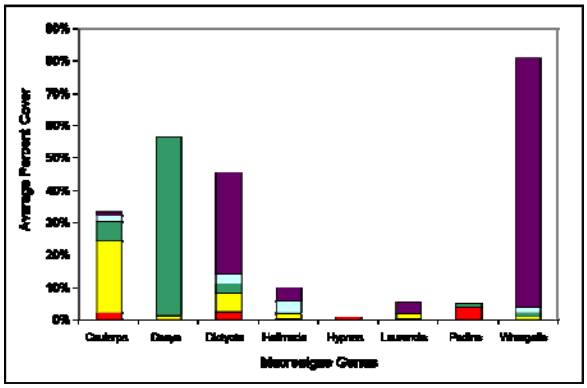


Figure 6: Average percent cover of dominant macroalgae genera over time.

The octocoral and stony coral community were also analyzed to determine the initial colonizers and successive genera (Fig. 7).

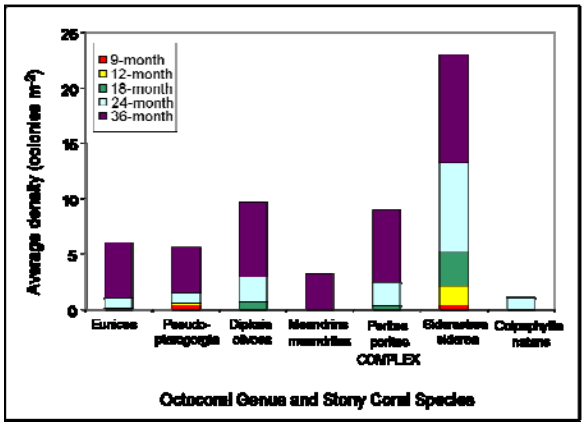


Figure 7: Density of dominant octocoral genera and coral species over time.

Overall two soft coral genera dominated with *Pseudopterogorgia* spp. initially recorded at the 9-month monitoring event while *Eunicea* spp. were recorded, at a very low density, during the 12-month monitoring event. Of the scleractinians, *Siderastrea siderea* was the only pioneer species recorded during the 9-month survey. *Siderastrea* spp. are considered one of the most stress tolerant species found in southeast Florida (Lirman et al. 2002). Four additional scleractinian species dominated during the subsequent monitoring events including *Diploria clivosa, Meandrina meandrites, Porites porites* complex, and *Colpophyllia natans*. *D. clivosa* and *P. porites* were initially recorded at the 18-month survey, while *C. natans* was first recorded at the 24-month event. *M. meandrites* was only documented during the 36-month post-deployment monitoring survey.

Discussion

The three year study of benthic community development showed a series of biotic changes over time. Initial colonizers on the Broward County AR system consisted of typical fouling organisms such as bryozoans, hydroids, sponges and tunicates. Bryozoans and hydroids are known fouling organisms which are often replaced over time in biotic succession (Abdel-Aleem 1957; Otsuka and Dauer 1982; Palmer-Zwahlen and Aseltine 1994). As the AR substratum was biologically altered and became more complex over time, the benthic community differentiated further. Results demonstrate that a large percentage of these pioneering organisms were replaced by more persistent and biologically complex organisms, *i.e.* scleractinians and octocorals, as the AR "soak" time increased. This ecological successional pattern is supported by overall increasing trends in diversity indices.

As of 36-month post-deployment, bryozoan percent cover decreased by 4%, tunicate percent cover decreased by 2.6%, sponge percent cover decreased by 10.4%, and hydroid percent cover decreased by 1.7%.

Fouling organisms are essential in elevating the habitat complexity of AR systems, thus making it suitable for coral settlement (Perkol-Finkel and Benayahu 2005; Schuhmacher 1988) and a large increase in octocoral and coral density over time was evident. However, while this increase may be attributed to scleractinians' preference to settle on a complex substratum rather than simple one (Carleton and Sammarco 1987; Guichard et al. 2001), coral larvae tend to remain inconspicuous upon settlement (Edmunds 2000). Hence, coral recruits present at the 9-month monitoring survey may not have been visually conspicuous and were, therefore, not accounted for until the 12-month post-deployment survey.

Results indicate a shift in community composition over time; however, a classic directional successional pattern was not evident. As the MDS ordination of the biotic community over time suggests, and the ANOSIM results confirm, a strong similarity between the 12- and 24-month assemblages occurred, while a strong difference was detected between the 24- and 36-month assemblages.

Considering that succession did not proceed in a classic directional pattern beyond 18 months post-deployment may be due to the frequent physical disturbances present in the area during 2005. Two hurricanes affected the Broward County coastline immediately preceding the 24-month monitoring

survey. Both Hurricane Dennis and Katrina elevated wave frequency and heights resulting in extreme impacts to the nearshore coastline during July and August 2005. This included intense sand inundation and scouring. Eradication of the existing biota on the AR system may be correlated to these disturbances including the significant decrease in the total macroalgae percent cover. Macroalgae cover increased consistently until the 24-month monitoring event when it was then reduced by a total of 9.9%. Macroalgae cover rebounded post-hurricane by a total of 12.8%, as observed during the 36-month monitoring survey.

Unlike macroalgae, stony coral average percent cover increased consistently throughout this study suggesting that the high relief of the AR system may have provided protection from burial associated with storm activity.

In summary, community structure exceeded an initial developmental stage. Colonization of the AR system investigated in this study can be explained only partially in terms of classic directional succession as physical disturbances impeded progress to a possible climax community.

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Effects of herbivore grazing on juvenile coral growth in the Gulf of Mexico

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Abstract. This study demonstrates how herbivore grazing and substrate variations influence coral recruitment patterns at the Flower Garden Banks, Gulf of Mexico, for two brooding corals, *Agaricia spp.* and *Porites spp.* Three herbivore treatments and two tile textures and orientations were tested. Herbivore grazing significantly affected coral growth for *Agaricia,* but had no effect on *Porites*. Data suggest that grazing of *Diadema antillarum* and *Cerithium litteratum* negatively affected growth of juvenile *Agaricia* since smaller colonies were observed in herbivore treatments ($p=0.0034$). *Agaricia* recruits were larger on rough textured tiles ($p=0.0054$), but tile texture did not affect *Porites* size. Tile orientation preference differed between species, with *Agaricia* having larger and more colonies on vertical tiles ($p<0.0001$) whereas *Porites* colonies were larger and more numerous on horizontal tiles ($p=0.0053$). Our findings suggest that recruitment and growth of corals varies considerably between species and that this needs to be considered when developing reef conservation strategies.

Keywords: Herbivory, coral recruitment, *Diadema antillarum,* phase shifts

Introduction

Competition for primary space plays a major structuring role on coral reefs (Lang and Chornesky 1990) and phase shifts from coral to algal dominated communities highlight the importance of this competition. Reports of declining reef resilience and phase shifts towards algal dominated communities appear to be increasing at an accelerated rate (Done 1991; McCook et al. 2001; Hughes et al. 2003, 2007; Aronson and Precht 2006). Caribbean reefs have experienced an average of 40% coral loss since the 1970's (Gardner et al. 2003) and exhibit little recovery as recruitment rates decrease. Many factors affect coral recruitment, however algal competition appears to play a significant role in inhibiting recruitment and growth (McCook et al. 2001; Lirman 2001; Jomba and McCook 2002; Birrell et al. 2005; Vermeij 2006).

Grazing has been suggested as a tool to mitigate coral recruitment since herbivore exclusion experiments have demonstrated that reduced grazing causes declines in coral cover (e.g. Lirman 2001) and increasing grazing creates more suitable substrate on which corals can recruit (Omori *et al*. 2006). Caribbean reef communities appear to be highly influenced by the echinoid species, *Diadema antillarum* (Sammarco 1980; Carpenter 1988, Carpenter and Edmunds 2006), which was profoundly demonstrated in 1982 when a natural, species-specific pathogen induced a mass mortality of *D. antillarum* across the entire Caribbean. Mortality rates reaching 99% were observed (Lessios et al. 1984) and reefs were taken over by robust algal species, which caused a decrease in coral recruitment (Hughes et al. 1989). The recovery of *D. antillarum* has been remarkably slow; however, an increase in juvenile coral recruitment has been associated with urchin resurgence in Jamaica suggesting that these herbivores are a keystone species for coral growth (Carpenter and Edmunds 2006).

The Flower Garden Banks National Marine Sanctuary (FGB) (Fig.1) is an ideal area to study coral recruitment and competition, not only for its pristine conditions, but also for its relatively low species diversity (Gittings, 1992), which offers a simpler approach to understanding the ecology of the system. This study investigates how herbivores influence early coral recruitment and growth in the Gulf of Mexico.

Figure 1: Map the Gulf of Mexico showing the location of the Flower Garden Banks National Marine Sanctuary, 115 miles south of the Texas/ Louisiana border.

Recruitment rate variations can have profound repercussions on the management of coral reefs and low coral recruitment rates currently being observed on Caribbean reefs are likely to result in slow reef recovery after natural or anthropogenic disturbances (Hughes et al. 1999). Using herbivorous species to promote recruitment has been suggested as a solution to reduce the long-term decline of coral communities (Hughes et al. 2005; McCook et al. 2001).

Material and Methods

Experimental setup

A 6m x 6m x 0.6m galvanized steel platform was constructed in June 2007 on a sand patch at 24m depth on the East FGB (27º54'33.0"N, 93º35'59.7"W, Fig.2) to provide a controlled environment to test the effects of herbivores and tile texture and orientation on coral recruitment and growth. Nine fiberglass bins (117cm x 36cm x 25cm), each randomly assigned to one of three treatments (1. *Diadema antillarum*; 2. *Cerithium litteratum*; 3. No herbivore control), were attached. Twelve quarry tiles (15cm x 15cm) were suspended within each bin and served as settlement substrata. Tiles had one smooth side and one grooved side (Smooth, Rough) and two orientations (Vertical, Horizontal) were tested. Herbivore densities within treatments were determined by conducting herbivore censuses of the surrounding reef area and two *Diadema antillarum* and 50 *Cerithium litteratum* per bin were used. All animals were collected at night using SCUBA and retained within bins using 1.3cm^2 wire mesh.

Figure 2: Coral recruitment platform constructed on a sand patch on the East Flower Garden Banks at 24m depth.

Data analysis:

In April 2008, after nine months of natural recruitment, four tiles from each bin were photographed using a high-resolution digital underwater camera with strobe. All recruits (live and dead) were divided by genus (*Porites, Agaricia*) and total polyp/colony area (mm^2) was directly measured in ImageJ 10.2 and compared between herbivore treatments, tile textures and tile orientations. Coral size data were log-transformed to satisfy the assumptions of normality (Shapiro-Wilk) and equal variances for parametric statistical tests. Only main effects are presented here as no significance was observed for interaction terms. All statistics and graphics were performed in JMP 7.0.2.

Results

Herbivore treatment

Herbivory significantly affected colony size for *Agaricia* (p=0.0034) but not *Porites* (p=0.4445, Fig.3B) (Table 1). *Agaricia* recruits were the smallest in the sea urchin treatment, larger in the mollusk treatment and the control treatment had the largest recruits (Table 2, Fig.3A).

Table 1: Analysis of variance (ANOVA) table indicating variation coral size (mm^2) (log-transformed) for *Agaricia spp.* and *Porites spp.*

Source	DF	F Ratio	P
Agaricia spp.			
Treatment	2	15.46	0.0034*
Orientation	1	42.16	<.0001*
Texture	1	7.82	0.0054*
Porites spp.			
Treatment	2	0.82	0.4445
Orientation	1	8.32	0.0053*
Texture	1	0.42	0.8393

*Indicates significant difference α=0.05

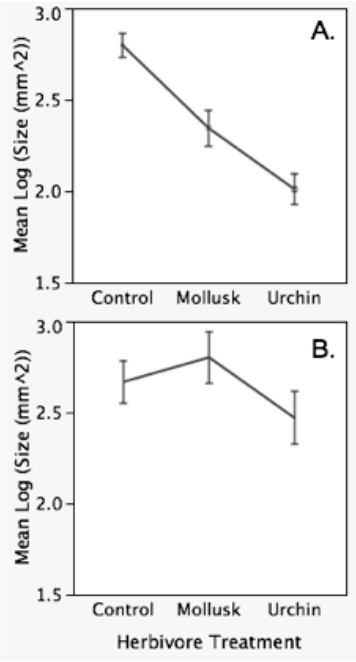

Figure 3: Variations in mean coral size (mm^2) between three herbivore treatments. A. *Agaricia spp.* (n=489) B. *Porites spp.* (n=76) Figure represents log-transformed data and standard error bars.

Table 2: Tukey's Studentized Range (HSD) test for significant differences in coral size (mm^2) (log-transformed) between different herbivore treatments.

	Tukey Grouping*	Least Sq Mean	Std Error	n
Agaricia α=0.05				**489**
Control	A	2.56	0.10	175
Mollusk	B	2.03	0.11	149
Urchin	C	1.77	0.10	165
Porites α=0.05				**78**
Control	A	2.33	0.20	29
Mollusk	A	2.65	0.20	31
Urchin	A	2.31	0.32	18

*Levels not connected by the same letter are significantly different

Tile texture

Tile texture had a significant effect on coral size for *Agaricia* (p=0.0054), however there was no texture effect for *Porites* (p=0.8393, Fig.4B) (Table 1). Student's t-tests demonstrated that *Agaricia* recruits on rough surfaces were significantly larger than those growing on smooth surfaces and there were also approximately three times as many recruits on rough surfaces (Table 3, Fig.4A)

Table 3: Student's t-test results for differences in coral size (mm^2) (log-transformed) between different tile textures and orientations.

	Least Sq Mean	Std Error	n
Agaricia			**489**
TEXTURE*			
Smooth	1.97	0.10	127
Rough	2.29	0.06	362
ORIENTATION*			
Horizontal	1.74	0.10	131
Vertical	2.51	0.07	358
Porites			**78**
TEXTURE			
Smooth	2.46	0.28	12
Rough	2.40	0.11	66
ORIENTATION*			
Horizontal	2.88	0.12	63
Vertical	1.98	0.27	15

*Indicates significant difference α=0.05

Orientation

Tile orientation significantly affected colony size for both *Agaricia* (p<0.0001) and *Porites* (p=0.0053) (Table 1), however, opposite trends were observed. *Agaricia* colonies were larger on vertical tiles and were approximately 2.5 more numerous on vertical tiles (Table 3, Fig.5A). *Porites* colonies were larger and over four times more numerous on horizontal tiles (Table 3, Fig.5B).

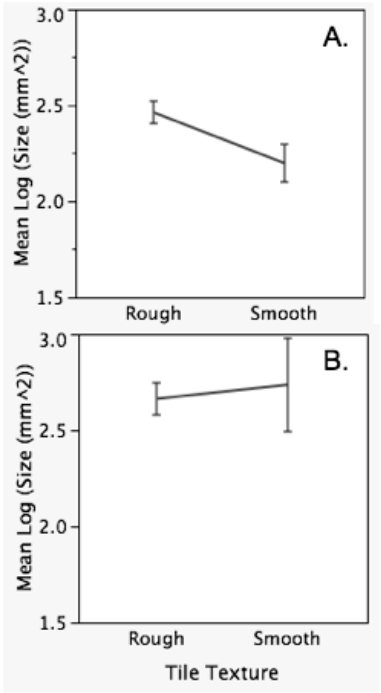

Figure 4: Variations in mean coral size (mm^2) between two tile textures. A. *Agaricia spp.* (n=489) B. *Porites spp.* (n=76) Figure represents log-transformed data and standard error bars.

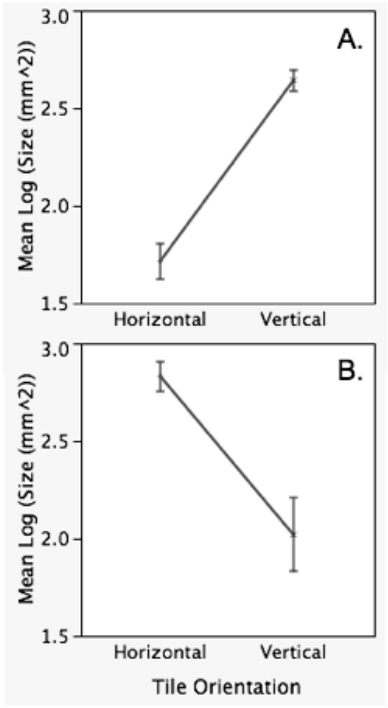

Figure 5: Variations in mean coral size (mm^2) between two tile orientations. A. *Agaricia spp.* (n=489) B. *Porites spp.* (n=76) Figure represents log-transformed data and standard error bars.

Discussion

This study on juvenile coral growth at the FGB demonstrates that herbivore and substratum variations can have significant effects on recruitment and that these effects vary between coral species. Two types of corals were observed to recruit, *Agaricia* and *Porites.* Both corals are brooders, where fertilization occurs within the polyp and released larvae are competent to settle almost immediately (Harrison et al. 1984). It is important to note that although these brooding coral species observed here are common on the FGB, they are not the dominant coral species. Coral cover at the FGB is dominated by other scleractinian corals, which reproduce annually in mass spawning events. The results observed here are representative of only one site in the Gulf of Mexico and caution needs to be taken when comparing these results to other coral species and reefs.

Coral recruitment is variable and larvae actively choose suitable substrate on which to settle. Space pre-emption by other benthic organisms can therefore lead to a decrease in local coral recruitment (Vermeij 2006). Research suggests that corals have inferior competitive capabilities and slower growth rates relative to other benthic organisms such as algae, which have been shown to smother young coral recruits (Miller and Hay 1998; Connell 1997; Edmunds and Carpenter 2001; Vermeij 2006). The top-down control of herbivore grazing on algal growth has been previously demonstrated (Carpenter and Pace 1997; McCook 1999) leading to the general hypothesis that herbivores may reduce competition, thereby increasing coral growth.

In this study we found that urchin grazing negatively affected coral growth for *Agaricia* but had no significant effect on *Porites* (Fig 3AB). Birrell et al. (2005) found that coral recruits almost exclusively preferred to settle on surfaces free from sediment and algae. Although urchin grazing has been correlated with increased coral recruitment (Carpenter and Edmunds 2006), we found that coral colony growth was significantly decreased in the sea urchin treatment, indicating that our experimental sea urchin levels resulted in destructive overgrazing. Destructive overgrazing occurs when echinoid densities are high and algal grazing becomes competitive and abrasive causing a reduction in coral growth (Sammarco 1980). For *Agaricia,* coral recruits were the largest in the control, which was not expected as most herbivore exclusion experiments have shown that when algae are released from grazing pressures, algal growth and coral mortality both increase (Sammarco 1980; Tanner 1995; Lirman 2001; Jomba and McCook 2002; Birrell *et al*. 2005). These unpredicted control results are most likely due to invasive herbivores recruiting to the control bins over the winter months. Several sea urchins were observed to recruit into all experimental bins, however this recruitment is presumed to be equal across all treatments.

Substrate texture has been shown to have a strong effect on juvenile corals. Peterson et al. (2005) found corals demonstrated a strict preference towards grooves in tiles and Harrison and Wallace (1990) indicated settlement and metamorphosis were generally favored in crevices as well. In this study we demonstrated that texture preference might be species specific. *Agaricia* experienced higher growth rates on rough textured tiles while *Porites* showed no significant difference. However, both species had more recruits on rough textured tiles (Table 3) and this is most likely due to the grooves providing sanctuary for corals from overgrazing and overgrowth by other encrusting species.

Research suggests that scleractinian corals show clear preferences for substrate position. Babcock and Mundy (1996) found that coral settlement on the undersurfaces of tiles was much higher, while Vermeij (2006) found higher survival rates on vertical surfaces. In this study we found that the effect of tile orientation on coral growth was highly species specific. *Agaricia* preferred vertical tiles with almost three times as many corals on vertical tiles and significantly larger sizes as well (Fig.5A). *Porites,* however, showed a higher affinity for horizontal tiles with significantly larger individual sizes and over five times as many recruits (Fig.5B). Vermeij (2006) has suggested that variations in orientation preference may stem from differences in growth rates. Faster growing species prefer open, exposed habitats while slower growing species prefer cryptic habitats because the faster growing corals may be more dependent on the symbiotic zooxanthellae in their tissues. During storms sand accumulated on horizontal tiles, which may have affected recruitment.

The distribution and abundance of corals reflect the patterns of successful recruitment and long-term mortality. Coral settlement and growth is not random but dependent on factors such as light, water circulation, sedimentation, competition, predation and grazing (Rogers *et al.* 1984). Monitoring coral growth is important since mortality rates decrease considerably as coral recruits increase in size (Wilson and Harrison 2005). Since recruitment is crucial for coral population maintenance, any disruption in coral reproductive output will eventually lead to long-term declines (Hughes and Tanner 2000; Nozawa *et al.,* 2006). By gaining a more comprehensive understanding of coral recruitment and competition dynamics, research will offer important insights for conservation strategies and management decisions of coral reefs.

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Settlement behavior of *Acropora palmata* planulae: Effects of biofilm age and crustose coralline algal cover

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Abstract. The role of crustose coralline algae (CCA) and bacterial biofilms in the settlement induction of *Acropora palmata* larvae was tested with ceramic tiles conditioned in reef waters for different time periods. Larval settlement varied among tiles by conditioning time (P<0.001), with low settlement (11%) on unconditioned tiles and high settlement (72-87%) on tiles conditioned for 2, 8 and 9 weeks. Tile surface texture and orientation also affected settlement (P<0.001). Larvae of *A. palmata* preferred the undersides of tiles as conditioned in the field (78% of total settlement), compared to upper surfaces (8%) or Petri dish surfaces (14%). CCA cover increased with conditioning time (P<0.001) and differed by tile orientation (P<0.005), revealing a positive correlation between settlement and CCA cover on tile bottoms, but not tile tops. Terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes revealed that biofilm age, tile surface and tile orientation affected microbial community structure. Further, biofilms that induced settlement were characterized by bacterial populations distinct from non-inductive biofilm communities. Thus, we present additional evidence of the involvement of CCA and bacterial biofilm communities in the process of coral larval settlement, suggesting that complex interactions among multiple cues are involved in larval settlement choices.

Key words: *Acropora*, settlement cues, microbial community, T-RFLP, crustose coralline algae

Introduction

Scleractinian corals are the foundational organisms that provide coral reefs with the habitat complexity that makes them amongst the most biologically diverse of marine ecosystems. For coral reefs to form, many species of corals must dominate the reef substrate and occupy space over long evolutionary time periods. This must involve both long-lived adult corals and persistent high rates of coral recruitment success on new substrates following disturbances.

Coral recruitment begins with the attachment of a coral planula larva to reef substrate and is successful when the environment into which the larva settles supports its long term survivorship. Conditions favoring coral recruitment success include low competition with faster growing algae and encrusting invertebrates, promoted by high rates of grazing (Hughes 1994; Carpenter 1997; Hixon 1997; Birrell et al. 2005). However, coral larvae are structurally simple and have limited ability to sense the environment, except through chemosensory means.

The current view of coral settlement is that when competent planulae explore the reef substrate, contact with certain chemical cues triggers them to stop swimming, attach to the substrate and develop into the primary polyp (reviewed in Harrison and Wallace 1990). These cues are thought to be chemicals associated with or secreted by crustose coralline algae (CCA) or microbes growing on the substrate. Chemical extracts from certain species of CCA have been demonstrated to induce coral settlement more strongly than others, indicating preference and specificity for cues associated with those species (Morse et al. 1988, 1996; Morse and Morse 1991, Heyward and Negri 1999, Harrington et al. 2004). However, settlement experiments conducted on *Montastraea faveolata* and *Acropora palmata* revealed that most larvae settled near, rather than on, CCA patches (Szmant and Miller 2005). A more recent, detailed study found that over 80% of the larvae from these coral species attached onto microbial films (Nugues and Szmant in prep.), suggesting that microbial communities may be responsible for generating the settlement signal. Indeed, studies have demonstrated the induction of larval settlement by bacteria, including a cultured isolate from CCA (*Pseudoalteromonas* sp. strain A3, Negri et al. 2001) and diverse, naturally-occurring reef biofilm communities (Webster et al. 2004).

Determining the settlement cues of coral larvae is crucial to reef conservation and restoration efforts, allowing for the identification and targeted preservation of high recruitment reef niches, as well as, the direct application of replicated cues to coral nursery programs requiring induction of larval metamorphosis under laboratory conditions.

In this study, we used an experimental approach similar to that described by Webster et al. (2004) to determine the role of CCA and microbial biofilms in larval settlement induction. New substrates (ceramic tiles) were 'conditioned' in seawater to develop natural reef biofilms. The complexity of biotic communities that develop on new substrates increases with field conditioning time, as does their attractiveness to coral larvae (Webster et al. 2004). Tiles were aged from 0 to 9 weeks in a back reef lagoon and used in settlement bioassays. Terminal restriction fragment length polymorphisms (T-RFLP) analysis, a popular culture-independent microbial profiling technique (Schütte et al. 2008), was used to characterize the community structure of microbial biofilms. We present here the correlations between settlement of the larvae of the Caribbean reef coral, *A. palmata*, and these microbial signatures, as well as, the amount of CCA colonizing the tiles.

Material and Methods

Settlement tiles: orientation and texture
Ceramic tiles (high-fired clay, Daltile®) attached to plastic racks on cement blocks were pre-conditioned in the field at 4 m for 2, 4, 6, 8 and 9 weeks. Tiles consisted of a glazed, flat surface and an unglazed, dimpled surface. For each time point, 24 tiles were deployed glazed-side up and 24 glazed-side down. Thus, there were 4 possible combinations of tile surface texture and orientation during field conditioning. Tiles were collected simultaneously in individual bags the day settlement experiments were conducted. Control tiles were soaked in 5µm filtered seawater (FSW) overnight.

Gamete collection and larval rearing
Gamete bundles were released by *A. palmata* colonies during the night of September 1, 2007. Spawn from multiple colonies were collected, cross-fertilized and reared to the planulae stage (Szmant and Miller 2005).

Settlement assay
Large Petri dishes (150 mm x 25 mm) were filled with 200 mL FSW at 29 °C and one tile of each orientation – glazed-side up and glazed-side down, as oriented in the field – were added to each dish. Twenty competent *A. palmata* larvae were added to each dish, with 5 replicate dishes per time treatment. After 48 hours, settled larvae were enumerated on each tile using a dissecting microscope. Following settlement counts, digital photographs were taken of each tile face and sterile cotton swabs were used to sample biofilm communities. Paired T-tests, a 2-way ANOVA and a nested ANOVA (tile texture and tile orientation nested with conditioning time) were run to determine the effects of conditioning time, tile texture and tile orientation on larval settlement using SigmaPlot 11.0 and Statistica (1998 version).

Crustose coralline algae quantification
Digital photographs were processed using ImageJ software (NIH). Quantification of CCA was only possible on glazed tile surfaces, where contrast was high between CCA patches and the tile background. Photographs were cropped to fit the tile surface, CCA patches were defined as regions of interest, and black and white mask images were created. CCA cover was calculated by measuring the dark area of the mask images. A 2-way ANOVA was run to determine the effects of conditioning time and tile orientation on CCA cover. Linear regression analyses were performed to compare CCA cover and larval settlement rates on each tile face (SigmaPlot 11.0).

DNA extraction and amplification
Community DNA was extracted from biofilm swabs preserved in RNAlater (Ambion) using the PUREGENE kit (Gentra Systems) and purified using the Wizard DNA Clean-Up System (Promega). Universal bacterial primers 8F-FAM (Reysenbach et al 1994) and 1522R (Martinez-Marcia et al 1995) were used for amplification of partial 16S rRNA sequences. Total PCR reaction volume was 50 µl, including 15 pmol of the forward primer, 10 pmol of the reverse primer, 25 µl GoTaq® Mastermix (Promega) and 2 µl DNA template. Thermocycler conditions consisted of an initial denaturing time of 5 min at 85 °C, then 35 cycles of 0.75 min at 94 °C, 1 min at 55 °C, and 1.5 min at 72 °C, with a final extension time of 10 min at 72 °C. For each sample, PCR products from 3 separate reactions were combined and gel-purified using the Wizard SV Gel Clean-Up System (Promega). PCR products were quantified using the Quant-iT Qubit® kit (Invitrogen).

Restriction enzyme digestion and T-RFLP analysis
Approximately 100 ng of purified PCR products were digested with the restriction endonucleases *HaeIII* and *MspI*. Total reaction volumes for *HaeIII* digests was 20 µl, including 17.3 µl PCR product, 0.2 µl BSA, 2 µl Buffer C and 0.5 µl enzyme (Promega). Total reaction volume for *MspI* digests was 20 µl, including 17.3 µl PCR product, 0.2 µl BSA, 2 µl NEBuffer 2 and 0.5 µl enzyme (New England Biolabs). All digests were incubated at 37 °C for 12 hours. Immediately following digestion, samples were precipitated with 75% isopropanol and dried (SpeedVac, LabConco). To each sample, 10 µl formamide and 0.5 µl ROX size standard (PE Applied Biosystems) were added. Samples were denatured at 94 °C for 2 min and immediately cooled on ice for 2

min. Samples were analyzed on an automated sequencer (ABI377) with the program GeneScan (PE Applied Biosystems). The length of individual fluorescently-labeled terminal-restriction fragments (T-RFs) was determined by comparison with TAMRA size standards (Genescan™). Raw T-RFLP peak profiles were standardized using a proportional threshold of total fluorescence (Osborne et al 2006) and compared across samples using T-Align (Smith et al 2005). Peak profiles were standardized using relative abundance and peaks with areas <1% of total fluorescence were discarded as background noise.

Non-metric multi-dimensional scaling plots

To assess the similarity of bacterial community structures, Bray-Curtis similarity matrices were constructed using square root transformations of relative abundance T-RFLP data. To visually compare bacterial community similarity across samples and factors, non-metric multi-dimensional scaling (MDS) plots were constructed. Analyses of similarity (ANOSIMs) assessed the statistical significance of similarity among biofilm bacterial communities by the factors tile age, tile surface, tile orientation, and larval settlement. MDS plots and ANOSIM calculations were performed using PRIMER v5.1.2 (Plymouth Marine Laboratory, UK).

Results

Settlement assay

Larval settlement varied significantly over conditioning periods (P<0.001), with low settlement on unconditioned tiles, moderate settlement on tiles conditioned for 4 and 6 weeks, and high settlement on tiles conditioned for 8 and 9 weeks (Fig. 1). Notably, 2-week old biofilms induced settlement levels (72%) similar to the oldest biofilms (8 and 9 weeks), while settlement rates dropped in 4 and 6 week old biofilms.

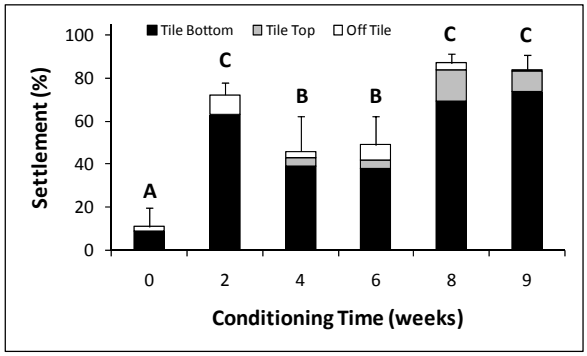

Figure 1. Effect of conditioning time on settlement of *A. palmata* planulae. Black bars represent tile surfaces facing upwards, gray bars tile surfaces facing downwards, and white bars non-tile substrate (Petri dish). Error bars represent 1SD and letters denote significant (P<0.05) differences in total settlement by treatments.

Within conditioning treatments, settlement rates were significantly affected by tile orientation (P<0.05) and tile surface (P<0.05). Total settlement of *A. palmata* larvae showed a clear preference for tile bottoms (78%) compared to tile tops (8%), as oriented in the field, with low settlement (14%) found off tiles.

Crustose coralline algal cover

CCA cover on settlement tiles increased significantly with longer conditioning periods (P<0.001); however, variation within treatments was large. Settlement tiles conditioned for greater than 4 weeks exhibited higher levels of CCA cover than control tiles and tiles conditioned for 2 weeks. CCA cover also varied significantly by tile orientation (P<0.005), with higher cover on tile tops compared to tile bottoms; however, pairwise comparisons within conditioning treatments were only significant for 6-week tiles (P<0.001; Fig. 2).

Linear regression analysis revealed that CCA cover on tile bottoms was positively correlated with larval

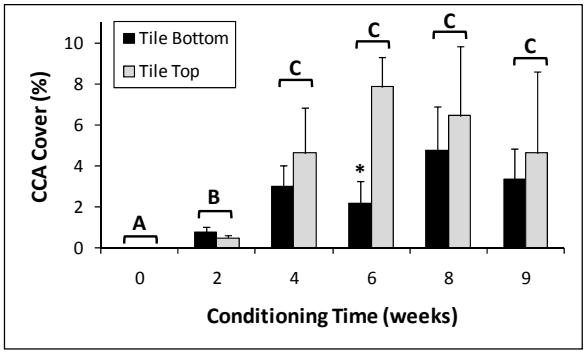

Figure 2. Effect of conditioning time and tile orientation on CCA cover. Black bars represent tile surfaces oriented downwards (bottom) and gray bars tile surfaces oriented upwards (top). Letters indicate significant (P<0.05) pairwise differences among conditioning treatments. Asterisks denote significant (P<0.05) differences among tile bottoms and tile tops within conditioning treatments. Error bars represent 1 SD.

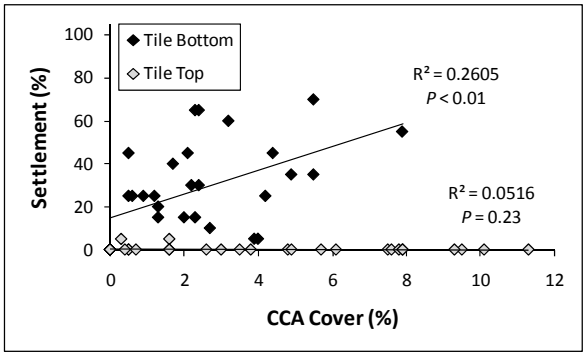

Figure 3. Effect of CCA cover and tile orientation on settlement of *A. palmata* planulae. Dark diamonds denote tile surfaces oriented downward (bottoms) and gray diamonds surfaces oriented upward (tops). Regression lines are shown for tile bottoms and tops.

settlement (P<0.01) and explained 26% of the variation in settlement by *A. palmata* larvae on tile bottoms. However, larval settlement on tile tops was not correlated with CCA cover (P=0.23), despite exhibiting a similar range of CCA cover found on tile bottoms (Fig. 3).

Microbial diversity and biofilm community structure

A total of 47 unique terminal restriction fragments (T-RFs) were identified from 27 T-RFLP profiles analyzed using the restriction endonuclease *MspI*; only 24 T-RFs were identified from 27 T-RFLP profiles using *HaeIII*. Lower resolution with *HaeIII* digestion is likely attributable to excessive digestion of 16S gene sequences, evidenced by short (<60bp), saturated peaks in chromatographs (data not shown).

ANOSIMs revealed distinctions among biofilm bacterial communities based on conditioning periods, tile surface, and tile orientation (Table 1). Distinct bacterial communities were also recovered from tiles inducing settlement compared to non-inducing tiles (*MspI* data). MDS plots exhibited low stress values, indicating congruence between MDS plot and similarity matrix distances. Samples clustered by factors with some overlap among factor levels, representative of the modest R-statistic values reported. MDS plots constructed from T-RFLP analysis with *MspI* showed clearer distinctions among factors compared to *HaeIII* (Fig. 4).

Table 1. ANOSIM summary statistics (R-statistic and *P*-value) for T-RFLP data recovered using *HaeIII* and *MspI*.

	HaeIII		***MspI***	
Factor	R-statistic	*P*-value	R-statistic	*P*-value
Age	0.217	0.002	0.192	0.009
Surface	0.069	0.046	0.210	0.003
Orientation	0.171	0.002	0.305	0.001
Settlement	0.087	0.099	0.186	0.013

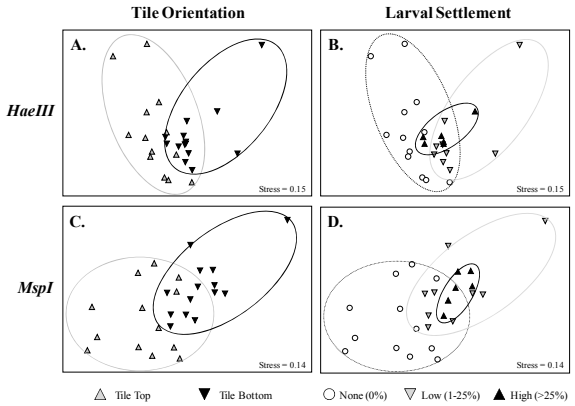

Figure 4. Non-metric multi-dimensional scaling ordination of biofilm bacterial communities recovered by T-RFLP analysis with the restriction endonucleases (A, B) *HaeIII* and (C, D) *MspI*. Data are grouped by (A, C) tile orientation and (B, D) larval settlement induction levels; circles encompass all samples by each factor level.

Discussion

Settlement behavior of *A. palmata* planulae was significantly affected by the conditioning periods of settlement tiles. Both CCA cover and biofilm bacterial communities varied significantly across conditioning treatments, but neither could consistently explain the observed larval settlement preferences. CCA cover correlated with settlement, but only on tiles undersides and accounted for only 26% of settlement variability. Previous work has also reported preferential settlement of coral larvae on the undersides of field conditioned tiles (Babcock and Mundy 1996; Raimondi and Morse 2000; Szmant and Miller 2005; Szmant in prep.). Other studies have reported little effect of CCA presence on larval settlement (Petersen et al. 2005) and settlement of coral planulae in the absence of CCA cues (Negri et al. 2001; Webster et al. 2004). While CCA and non-coralline crustose red algae have been implicated in the induction of coral larval settlement (e.g., Heyward & Negri 1999), settlement cues of crustose algae vary with the algal species and settlement response with different coral species (Golbuu and Richmond 2007). In this study, CCA that colonized settlement tiles may not have represented 'inductive' algal species for *A. palmata* larvae.

Another hypothesis is that the CCA function as part of a multi-cue cascade that leads planulae to settle and metamorphose, in which physical conditions (e.g., low light intensity) or other chemical cues initially attracted larvae to tile undersides, where close or direct contact with CCA surfaces then signals an attractive habitat to the planulae. Indeed, phototactic behavior by coral larvae and increased settlement on tile bottoms under high light intensities has been reported (Mundy and Babcock 1996). In addition, evidence for synergistic interactions among multiple chemical cues derived from CCA that enhance coral larval settlement has been recently reported (Kitamura et al 2007). Clearly, some attribute of tile undersides proved a stronger larval attractant than CCA alone, as CCA were similarly abundant on tile tops where very little to no settlement occurred.

Microbial biofilms play a key role in the settlement induction of many cnidarian larvae (Müller and Leitz 2002), including scleractinian corals (Negri et al. 2001). In fact, 'conditioning' of settlement tiles for microbial biofilms is routinely performed prior to coral larvae bioassays and usually required to achieve high larval settlement. Despite the crucial role of biofilms in larval settlement, few studies have investigated the bacterial community structures established by 'conditioning' (Webster et al. 2004). One recent comprehensive study employed culture-independent molecular and electron microscopy methods to characterize biofilm communities

inhabiting conditioned slides used in settlement assays (Webster et al. 2004). The authors reported no differences in relative densities of major bacterial lineages between biofilms that induced settlement and those that did not, and suggested metamorphic cues may be associated with particular bacteria species (Webster et al. 2004). Similarly, T-RFLP analysis of biofilm bacteria in this study revealed community-level differences among microbial films based on conditioning periods, tile surfaces and tile orientations. Further, distinct biofilm communities were recovered from tiles that induced settlement compared to those that did not. These results further highlight the complex nature of larvae-bacteria interactions and suggest multiple sensory cues from biofilm communities affect settlement choices by coral larvae.

Determining the environmental cues that dictate coral larvae settlement is essential to understanding how reefs will respond to and recover from reef degradation and is crucial for successful reef restoration programs. Loss of critical settlement cues from reef biota will result in continued, precipitous ecosystem declines by reducing coral recruitment at impacted sites. Identification of settlement cues may offer insight into preservation of biotic components that enhance reef recovery and aid restoration efforts, by providing a stock of metamorphic cues to enhance larval settlement under artificial conditions and the production of juvenile corals for transplantation to degraded reefs. The roles of some reef organisms (e.g., CCA) have been commonly targeted; however, other key organisms (e.g., bacteria) require further investigation. Additional studies targeting the role of coral reef microbial communities in larval settlement induction are required to fully elucidate the cues that attract new recruits and thus sustain reef ecosystems.

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Multivariate responses of the coral reef fish community to artificial structures and coral transplants

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Abstract. The effects of artificial structures and coral transplants on fish abundance, number of species, and community composition were examined during a two-year study at three locations in North Sulawesi, Indonesia. Three experimental treatments (10 x 10 m rubble plots (a.) left untreated, (b.) covered with concrete structures, and (c.) covered with a combination of structures and coral fragments) were monitored using monthly underwater visual censuses. An additional census of adjacent plots in the ambient reef served as comparison with the natural reef fish community. The effect of treatments differed between experimental sites. At the site with the lowest coral cover and structural complexity in the ambient reef, fish community responses were most marked, with an 8-fold increase in fish abundance in the Structures + Corals treatment compared to the untreated rubble plot. At the other two sites, abundance and number of species in the experimental treatments did not surpass that of the natural plots. At all locations, multivariate dispersion of the fish community samples decreased with increasing complexity of the experimental plots. The results underline the importance of reef context in the selection of appropriate restoration measures and show that results may differ depending on condition of the ambient reef.

Key words: coral transplantation, artificial reefs, Indonesia, fish community, restoration

Introduction

Coral reefs are complex, heterogeneous marine habitats mainly composed of biogenic carbonate structures that are deposited by hermatypic scleractinian corals and host numerous associated species of animals and plants (Vaughan 1919, Wells 1957). Fishes are among the most conspicuous reef dwellers. A wide range of fish species depend on live corals, e.g. for food or shelter (Patton 1976, Choat and Bellwood 1991, Cole et al. 2008). Fish abundance and species diversity are positively correlated with habitat complexity and amount of live coral cover (Risk 1972, Carpenter et al. 1982, Bell and Galzin 1984, Sano et al. 1984, Gratwicke and Speight 2005).

In response to the ongoing degradation of coral reefs world wide, the discipline of coral reef restoration has become increasingly established over the past two decades, although it remains largely in its infancy compared to the restoration of other ecosystems (Precht 2006, Edwards and Gomez 2007). Clark (2002) has cautioned that 'reef restoration is largely limited by incomplete knowledge on the ecosystem processes.'

Techniques frequently employed in coral reef restoration include the use of artificial reefs and transplantation of live corals (Jaap 2000, Clark 2002, Zimmer 2006), the latter currently being the most frequently used tool in active coral reef restoration (Edwards and Gomez 2007). However, despite the numerous studies on the link between live coral substrate and reef fishes, few workers have addressed the effects of coral transplantation on the reef fish community (Pamintuan et al. 1994, Cabaitan et al. 2008).

In this study, the effects of artificial structures alone and in combination with coral transplants on fish abundance and community composition were examined to gain a better understanding of the ecosystem processes pertaining to reef restoration.

Material and Methods

The present study was conducted at three locations in North Sulawesi, Indonesia (Fig. 1), between May 2005 and July 2007. The Gangga site was in the shallow upper reef slope in front of a dive resort, with low fishing pressure in the immediate vicinity of the experimental treatments, while the other two sites were at deeper sections of the reef slope (Table 1) and lay within the borders of Bunaken National Marine Park. At each site, three 10 x 10 m rubble plots were marked and cleared of remaining coral colonies and rocks, so that a flat rubble area remained. Plots lay at similar depths and were located between 5 and 20 m apart. At each site, the plots were randomly assigned to three treatments. Control plots (**C**) remained as empty rubble fields. In the other two treatments, the substrate was covered with 100 bamboo boards.

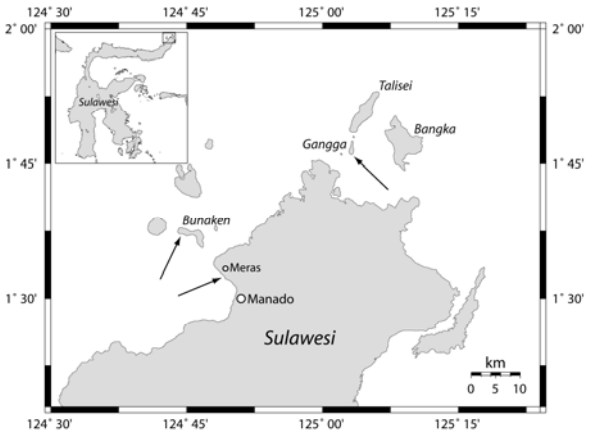

Figure 1: Map of northern Sulawesi, showing the three experimental sites. The location of the study area within Sulawesi is indicated on the inset map.

Artificial structures consisting of 5 concrete blocks each were then constructed on every second board in a chessboard pattern. In the Structures treatment (**S**), the remaining squares remained empty. In the Structures + Corals treatment (**S+C**), coral fragments were transplanted onto the empty bamboo boards between the structures (Fig. 2).

At each site, up to 2000 fragments of one species of branching *Acropora* were transplanted (Table 1). Species were selected based on abundance in the ambient reef. Each transplanted species occurred in large thickets in the vicinity of the recipient site. Additionally, fragments of *Pocillopora verrucosa* were transplanted at Gangga and Meras (Table 1). The fragments were distributed evenly among the boards and arranged at random on each board. A detailed description of the transplantation procedure is given by Ferse (2008).

Table 1: Depth of experimental plots, monitoring time frame and coral species transplanted for each site.

site	depth	monitoring	fragments transplanted*
Gangga	4-9 m	09.2005 – 05.2007	*Acropora yongei*: *n* = 1885 *Pocillopora verrucosa*: *n* = 475
Meras	12-20m	02.2006 – 06.2007	*Acropora formosa*: *n* = 1677 *Pocillopora verrucosa*: *n* = 378
Bunaken	12-19m	03.2006 – 05. 2007	*Acropora brueggemanni*: *n* = 1749

**n* = amount of surviving fragments at the time of the first visual census

A visual census of the fish community above the plots was conducted once per month, using three replicate counts carried out within the same day between 9:00 and 17:00 h. A SCUBA diver swam across each plot in four adjacent transects of 2.5 m width and 10 m length, counting and identifying all fishes present to a height of about 2.5 m above the substrate. Cryptic and pelagic species were excluded from the census. At the beginning of each count, horizontal visibility was estimated.

To compare the fish communities in the experimental plots with that of the ambient reef, per site two 10 x 10 m plots with substrate cover representative of the surrounding reef were marked in the vicinity of the experimental plots at similar depths, and a one-time census of the fish community with three replicate counts was carried out. Substrate composition in the natural reef plots, and detailed descriptions of the ambient reefs, are reported by Ferse (2008).

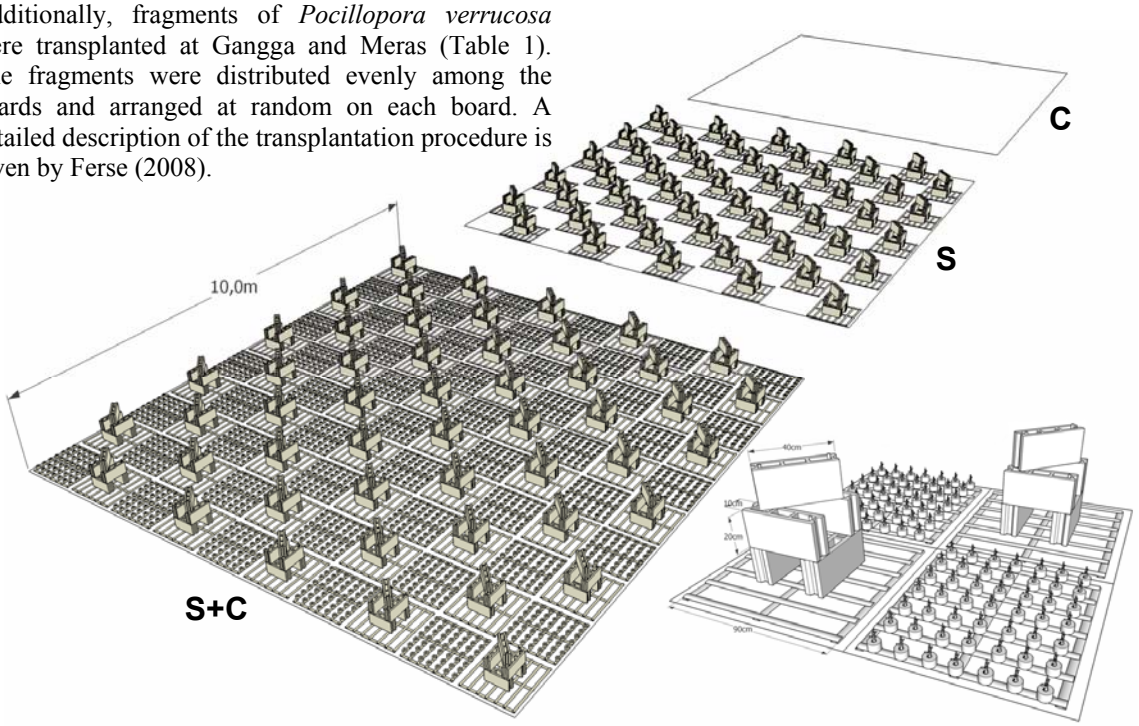

Figure 2: Sketch of the experimental set-up. In one treatment, artificial structures were interspersed with coral transplants (**S+C**, front), in a second one, only concrete structures were deployed (**S**, middle), and a third treatment consisted of a cleared coral rubble plot (**C**, back). A detailed drawing of the artificial structures and bamboo boards with attached coral transplants in shown on the lower right.

The effects of treatment on fish abundance and species diversity were assessed using a full-factorial general linear model, with time in days as independent variable and number of individuals and species as dependent variables. Underwater visibility was included as independent co-variable. Abundance data were square-root transformed to achieve normal distribution. Data from the natural reef plots were compared to data from the experimental plots taken at similar times using a one-way ANOVA. Where significant treatment effects were detected, a Tukey HSD post-hoc test at $\alpha = 0.05$ was performed to assess how treatments differed from each other. All univariate data analysis was carried out using JMP 7.0 (SAS Institute 2007).

For multivariate analysis of the fish community data, the program PRIMER 5.2.9 (Clarke and Gorley 2001) was used. Fish abundance data were fourth-root transformed and similarity of the samples was assessed using a zero-adjusted form of the Bray-Curtis coefficient (Clarke et al. 2006). To assess effects of treatments and sites on differences in the fish community composition, a crossed two-way multivariate analysis of similarities (ANOSIM) was used, and the relative multivariate dispersion of the community in the three treatments was analyzed for each site using the MVDISP routine.

Results

At all three sites, fish abundance increased markedly in the Structures + Corals treatment, while the increase in the Control plots was only slight (Fig. 3). The highest increase in abundance occurred at the shallow site (Gangga), where the number of individuals in the presence of coral transplants had increased almost 8-fold by the end of the experiment compared to the Control treatment. The linear model detected significant treatment effects (Table 2), and the post-hoc test confirmed significant differences between all three treatments.

Table 2: Results of the general linear model testing for effects of treatment, time and visibility on fish abundance at the three sites.

Test	Factor	*df*	*F*	*p*
Gangga				
ANOVA	Model	11,117	183.84	<0.0001
	($R^2 = 0.9453$)			
Effect Test	Treatment	2	646.52	<0.0001
	Time	1	458.03	<0.0001
	Visibility	1	0.68	n.s.
	Treatment*Time	2	62.52	<0.0001
	Visibility*Time	1	0.54	n.s.
	Visibility*Treatm.	2	8.15	0.0005
	Vis.*Time*Treatm.	2	2.79	n.s.
Meras				
ANOVA	Model	11,110	152.24	<0.0001
	($R^2 = 0.9384$)			
Effect Test	Treatment	2	640.31	<0.0001
	Time	1	89.03	<0.0001
	Visibility	1	34.32	<0.0001
	Treatment*Time	2	6.49	0.0022
	Visibility*Time	1	0.90	n.s.
	Visibility*Treatm.	2	2.35	n.s.
	Vis.*Time*Treatm.	2	10.87	<0.0001
Bunaken				
ANOVA	Model	11,105	35.54	<0.0001
	($R^2 = 0.7883$)			
Effect Test	Treatment	2	134.58	<0.0001
	Time	1	11.94	0.0008
	Visibility	1	21.17	<0.0001
	Treatment*Time	2	1.25	n.s.
	Visibility*Time	1	0.80	n.s.
	Visibility*Treatm.	2	6.75	0.0017
	Vis.*Time*Treatm.	2	0.94	n.s.

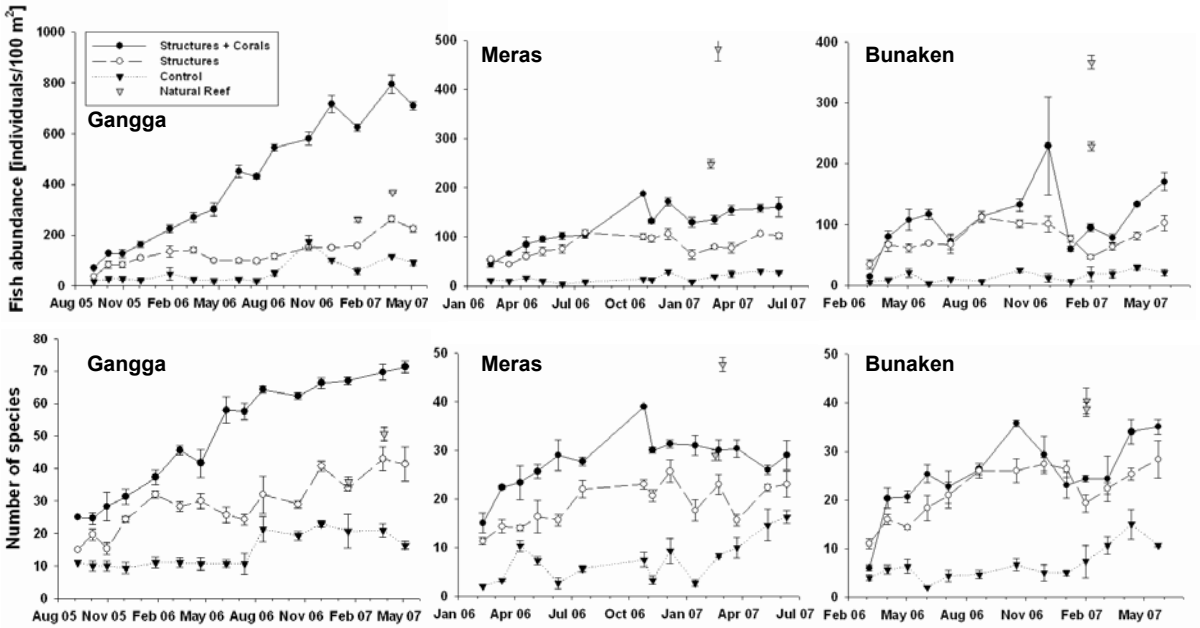

Figure 3: Fish abundance (above) and number of species (below) during consecutive censuses at the three locations (mean ± SE). The values observed during a comparative one-time census in two natural reef plots at each location are indicated by the gray triangles.

Fish abundance in the natural reef plots at Gangga was significantly lower than in the Structures + Corals treatment, but significantly higher than in the other two treatments (ANOVA, $F = 161.0741$, $p < 0.0001$, $df = 3,8$ and $F = 379.0192$, $p < 0.0001$, $df = 3,8$ for January 2007 and March 2007, respectively).

At the two deeper sites as well, the highest numbers of fishes occurred in the plots containing coral transplants. However, overall increases in abundance were less pronounced, and differences between the Structures and Structures + Corals treatments were not as marked as at Gangga (Fig. 3). Yet, the post-hoc test confirmed significant differences between all three treatments at both sites. Abundances in the natural reef plots were significantly higher than they were in the experimental plots at the time the ambient reef census was conducted (ANOVA, $F = 362.9089$, $p < 0.0001$, $df = 4,10$ and $F = 97.5778$, $p < 0.0001$, $df = 4,10$ for Meras and Bunaken, respectively).

The number of species followed similar trends as fish abundance at all sites. With few exceptions, species were most numerous in the presence of coral transplants, and the highest number of species was observed at Gangga (Fig. 3).

Table 3: Results of the general linear model testing for effects of treatment, time and visibility on number of species at the three sites.

Test	Factor	*df*	*F*	*p*
Gangga				
ANOVA	Model ($R^2 = 0.9272$)	11,117	135.65	<0.0001
Effect Test	Treatment	2	484.07	<0.0001
	Time	1	312.61	<0.0001
	Visibility	1	1.48	n.s.
	Treatment*Time	2	47.53	<0.0001
	Visibility*Time	1	0.83	n.s.
	Visibility*Treatm.	2	2.66	n.s.
	Vis.*Time*Treatm.	2	3.16	0.0461
Meras				
ANOVA	Model ($R^2 = 0.8724$)	11,110	68.38	<0.0001
Effect Test	Treatment	2	298.72	<0.0001
	Time	1	29.65	<0.0001
	Visibility	1	29.01	<0.0001
	Treatment*Time	2	0.36	n.s.
	Visibility*Time	1	4.15	0.0440
	Visibility*Treatm.	2	0.38	n.s.
	Vis.*Time*Treatm.	2	10.40	<0.0001
Bunaken				
ANOVA	Model ($R^2 = 0.7960$)	11,105	37.26	<0.0001
Effect Test	Treatment	2	141.82	<0.0001
	Time	1	54.55	<0.0001
	Visibility	1	4.91	0.0288
	Treatment*Time	2	2.23	n.s.
	Visibility*Time	1	0.23	n.s.
	Visibility*Treatm.	2	2.06	n.s.
	Vis.*Time*Treatm.	2	0.10	n.s.

Significant treatment effects were detected at all sites (Table 3), and post-hoc tests confirmed significant differences between the numbers of species in all treatments at each location. At Gangga, the amount of species in the natural reef plots did not differ significantly from that in the Structures treatment but was significantly different from the number of species in the other two treatments (ANOVA, $F = 48.9081$, $p < 0.0001$, $df = 3,8$ and $F = 59.5524$, $p < 0.0001$, $df = 3,8$ for January 2007 and March 2007, respectively).

Species number in one of the natural reef plots at Meras did not differ significantly from the number of species in the Structures + Corals plot. In the second natural plot at Meras, and in both natural plots at Bunaken, the amount of species observed was significantly higher than that found in the experimental plots at the same time (ANOVA, $F = 82.1000$, $p < 0.0001$, $df = 4,10$ and $F = 40.4127$, $p < 0.0001$, $df = 4,10$ for Meras and Bunaken, respectively).

Multivariate analysis of the fish community data revealed significant effects of treatment as well as location on community composition (Table 4).

Table 4: Results of a crossed two-way ANOSIM for effects of treatment and location using all experimental fish census samples.

Test	Factor	Test pairs	ρ
global	Treatment		0.643*
pairwise		Control, S + C	0.802*
		Control, Structures	0.751*
		S + C, Structures	0.564*
global	Location		0.830*
pairwise		Gangga, Meras	0.933*
		Gangga, Bunaken	0.891*
		Meras, Bunaken	0.664*

*significant at $p = 0.001$

As indicated by a multi-dimensional scaling plot of the averaged fish community data from all experimental plots (Fig. 4), the relative multivariate dispersion was highest in the Control treatment (MVDISP, relative dispersion 1.247, 1.688 and 1.489 at Gangga, Meras and Bunaken, respectively), while data from the Structures + Corals treatment were least dispersed (MVDISP, relative dispersion 0.734, 0.672 and 0.765 at Gangga, Meras and Bunaken, respectively).

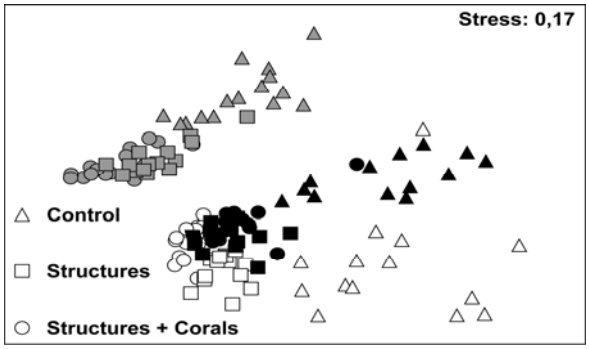

Figure 4: Non-metric Multi Dimensional Scaling (MDS) based on Bray-Curtis similarity of the average fish community data from all censuses of the experimental plots. **Gray:** Gangga, **White:** Meras, **Black:** Bunaken.

Discussion

Besides serving as food for corallivores, coral colonies can provide protection or additional habitat for fishes (e.g., Bell and Galzin 1984, Sano et al. 1984, Friedlander et al. 2003). As such, the observed positive effect of transplantation on fish abundance is not unexpected. However, fish community responses differed between the experimental sites. The most marked responses were observed at Gangga, where hard coral coverage in the ambient reef plots was less than 5 % (Ferse 2008). At Meras and Bunaken, where hard corals cover was between 13 and 29 % and the ambient reef was more heterogeneous than at Gangga, the effects of structures and coral transplants were less striking, and the values in the experimental plots remained below those in the ambient reef plots.

In previous studies, artificial reefs placed in areas of low complexity and little live coral cover have resulted in very high fish abundance and biomass (Randall 1963, Edwards and Clark 1993), while structures placed in biologically rich and structurally complex environments had values lower than the natural reef (Burchmore et al. 1985). Similarly, while the provision of structures and transplants was able to exert a significant impact on the fish community at Gangga, it appears that the experimental plots at the other two sites were not as attractive to fishes as the ambient reef area. This underlines that condition of the ambient reef should be a major consideration in the selection of appropriate restoration methods.

As indicated by a decrease in the relative multivariate dispersion of the community samples, structures and coral transplants aided in the establishment of a distinct fish community at all sites. Higher multivariate dispersion has been found in fish communities over degraded reef areas compared to healthy reef flats (Dawson Shepherd et al. 1992), and an analysis of relative dispersion may thus be an additional tool in judging the effects of restoration efforts.

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Recovery of injured giant barrel sponges, *Xestospongia muta*, offshore southeast Florida

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Abstract. Giant barrel sponges, *Xestospongia muta*, are abundant and important components of the southeast Florida reef system, and are frequently injured from anthropogenic and natural disturbances. There is limited information on the capacity of *X. muta* to recover from injury and on methods to reattach *X. muta* fragments. In late 2002, hundreds of barrel sponges offshore southeast Florida (Broward County) were accidentally injured during an authorized dredging operation. In early 2003, two to three months post-injury, 93% of 656 assessed injured sponges appeared to be recovering. In 2006, three years post-injury, nearly 90% of 114 monitored sponges continued to show signs of recovery. Growth rates were estimated by measuring sponge height above visual injury scars and ranged from 0.7 cm yr^{-1} to 6.0 cm yr^{-1}. Information on the artificially reattached fragments is limited but did show that *X. muta* fragments can reattach. This study provides evidence that *X. muta* in southeast Florida can naturally recover. Details on sponge size class associated recovery processes and growth were not collected due to event associated legal issues limiting the study. Studies to determine detailed growth rates and recovery success for different injury and restoration scenarios will further facilitate restoration decision making by resource managers.

Key Words: Barrel sponge, *Xestospongia muta*, recovery, reattachment, restoration

Introduction

The giant barrel sponge, *Xestospongia muta*, is an abundant, large, and important component of the southeast Florida reef system. Sponges are potentially very long-lived (McMurray et al. 2008) and have important ecological roles in reef ecosystems including filtering large volumes of water, contributing to habitat complexity, and increasing diversity with numerous associated infauna and microbial communities (Diaz and Rutzler 2001; Hentschel et al. 2006). Offshore Florida, *X. muta* colonies are often injured by marine construction activities (e.g. dredge projects), ship grounding and vessel anchoring events, line fishing, and storms (Schmahl 1999; Chiappone et al. 2002; Collier et al. 2007). Although *X. muta* has been shown to successfully heal small scale wounds, such as those caused by fish predation, few studies have focused on monitoring recovery from more severe injuries (Schmahl 1999; Walters and Pawlik 2005). There is also scant literature on the ability of *X. muta* fragments or dislodged barrels to reattach to the substrate. The limited availability of information on sponge fragment attachment success and natural recovery of severely injured sponges constrains the ability of resource managers to determine primary and compensatory restoration actions following injuries to *X. muta* populations (Jaap 2000; Collier et al. 2007).

In November 2002, during an inlet channel improvement project, steel tow cables were accidentally dragged over middle and outer reef areas offshore Broward County, southeast Florida. Damage to coral reef communities included the complete dislodgment of and various degrees of injury to hundreds of *X. muta* sponges. Injury assessment and primary restoration activities included the identification of injured barrel sponges and the reattachment of sheared sponge fragments. A monitoring effort was designed and included a representative sample of tagged injured sponges and transplanted fragments. Although legal issues (outside the scope of this paper) limited the monitoring effort, this study provides important information on the ability of *X. muta* to recover following severe injury and fragment attachment success.

Materials and Methods

Initial recovery

From December 2002 to February 2003, one to four months after the injury event, reef injury areas were assessed and mapped. As directed by the resource trustees (state of Florida), two types of additional assessments (transect and total-count) were conducted

to document initial *X. muta* recovery within a portion of the injury areas. Within seven mapped injury areas a total area of 900m² was assessed using 24, 0.75m x 50m, non-fixed, belt-quadrat transects. Four transects were completed within each of five of these injury areas and two transects were completed within two injury areas, and all transect locations were placed such that their entire length were within the mapped injury areas.

Within six total-count injury areas, which had a total injured area of 7,272m², all barrel sponges were counted and classified. For both of these rapid assessments, the trustees only requested information on the number of recovering sponges. No quantitative information on sponge sizes was collected. Injured sponges were classified as: *sheared*, with the entire barrel removed (Fig. 1), or *notched*, with only a portion of the barrel removed and most of the sponge remaining intact (Fig. 2). The condition of each sponge was also noted as recovering or not recovering. A recovering sponge was defined by the absence of necrotic tissue, darkening of the internal tissues exposed by the injury, and/or visible new growth.

Figure 1: Base of a sheared barrel sponge with a completely removed barrel and recently exposed internal tissues (white).

Fragment attachment

Also between December 2002 and February 2003, 401 *X. muta* fragments (sheared barrels) were collected and artificially attached to the substrate within the injury areas. Two methods were employed. In both methods, prior to attaching fragments, loose sediments, algae, and other material were removed to promote sponge growth onto the substrate. For the first method (210 of the 401 fragments) two lengths of stainless steel wire were crossed and draped over the fragment. The wire was pulled tight pressing the fragment against the substrate. The ends of the wire were secured to the substrate with cut nails. The second method (191 of the 401 fragments) utilized Portland cement to attach a portion of the fragment directly to the substrate. At the time of reattachment no data was collected on fragment size.

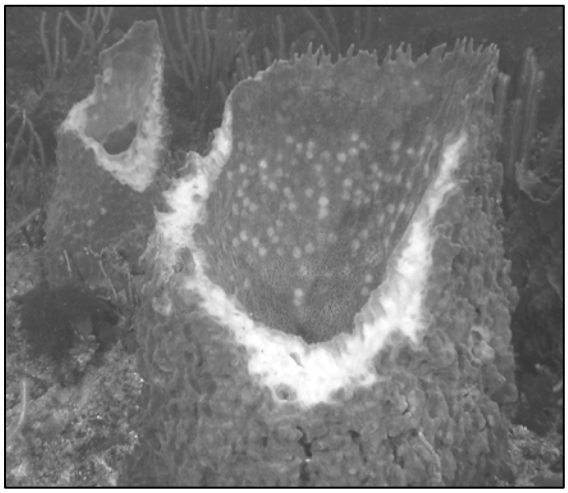

Figure 2: Two sponges with notched barrels and recently exposed internal tissues (white).

Long-term recovery

In March 2003, 11 monitoring zones were established to document long-term *X. muta* recovery within the injury areas. Four (zones 7, 9, 10, and 11) monitoring zones included injury areas within which the initial recovery assessments were completed. Zones were marked by a permanent center pin and included area around the pin defined by a radius of approximately 15m. Artificially reattached fragments and injured sponges within each zone were mapped and tagged. All sponges were alive and showed signs of recovery at the time of tagging. In December 2006 and January 2007, the 11 monitoring zones were visited and the tagged barrel sponges were assessed. For injured sponges and reattached fragments, condition was noted as recovering (alive) or missing (tag found but sponge missing). For the injured recovering sponges only, sponge height was recorded and new growth was estimated (when possible) by measuring (nearest cm) the height of tissue above the injury 'scar' (Fig. 3). Growth rates were estimated by dividing the measured new growth by the number of months between the injury and the time of measurement. This monthly growth rate was then multiplied by 12 for yearly growth. Growth had to be estimated due to the legal issues which stopped the project prior to collecting initial quantitative data in 2003 on sponge sizes. These same legal issues did not permit visits to the zones prior to the late 2006/early 2007 monitoring event.

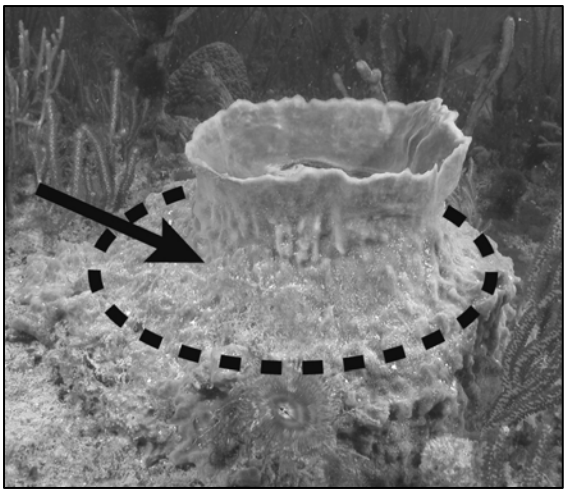

Figure 3: Injured barrel sponge in 2007 with the area of new growth (arrow) above a visible injury scar (dotted line).

Results

Initial recovery

Within the 24 transects 180 injured *X. muta* were identified with 13 classified as notched and 167 as sheared. Of these 180 sponges, 100% of the notched and 92% of the sheared (154 sponges) showed signs of recovery for a total initial recovery of 93% (167 sponges) (Table 1).

A total of 476 injured *X. muta* were identified within the six total-count injury areas. Forty-eight sponges were classified as notched and 428 as sheared. Forty-five notched sponges (95%) and 400 sheared sponges (93%) were recovering for a total recovery of 93% (Table 1).

Fragment attachment

Within the 11 monitoring zones, 26 reattached fragments were present and tagged. Six fragments were secured with cement and 20 with wire. During the 2006/2007 monitoring event, only five fragments (all wire) remained alive and had attached to the substrate (Fig. 4). The monitoring tags of the remaining 21 fragments (six secured with cement and 15 with wire) were found but the fragments were missing.

Figure 4: Artificially reattached barrel sponge fragment in 2007. Note the wire used to secure the fragment and the change in fragment growth orientation from vertical to horizontal.

Table 1: Initial recovery summary data for the seven transect and the six total-count (T-C) injury areas. Sponge totals for each assessment type and combined totals for both types are indicated (T = total sponges, N = notched sponges, S = sheared sponges).

Injury Area/	No. of	Area (m^2)			Injured			Recovering			Percent Recovering		
Assess. Type	Transects	Assessed	T	N	S	T	N	S	T	N	S
1 / Trans	4	150	33	4	29	31	4	27	94	100	93
2 / Trans	4	150	25	5	20	25	5	20	100	100	100
3 / Trans	4	150	19	1	18	17	1	16	90	100	89
4 / Trans	4	150	9	0	9	7	0	7	78	NA	78
5 / Trans	2	75	15	0	15	14	0	14	93	NA	93
6 / Trans	2	75	29	2	27	25	2	23	86	100	85
7 / Trans	4	150	50	1	49	48	1	47	96	100	96
Sub-Total	24	900	180	13	167	167	13	154	93	100	92
8 / T-C	NA	1,506	30	5	25	30	5	25	100	100	100
9 / T-C	NA	475	85	13	72	81	13	68	95	100	94
10 / T-C	NA	1,635	82	8	74	76	8	68	93	100	92
11 / T-C	NA	358	83	8	75	80	8	72	96	100	96
12 / T-C	NA	2,722	126	14	112	112	11	101	89	78.6	90
13 / T-C	NA	576	70	0	70	66	0	66	94	NA	94
Sub-Total	NA	7,272	476	48	428	445	45	400	94	94	94
		Total All	656	61	595	612	58	554	93	95	93

Long-term recovery

In 2003 within the 11 monitoring zones, 114 injured *X. muta* were tagged (Table 2). In late 2006/early 2007, 102 (90%) were found alive (recovering). Of the 11 monitoring zones, five had 100% recovery, and only one zone (zone 3) had less than 80% recovery (71.4%) (Table 2). Estimated new growth rates (recovery) of 101 surviving colonies ranged from 0.7 cm yr^{-1} to 6.0 cm yr^{-1}, with a mean of 2.78 cm yr^{-1} (± 0.11 SEM). It was noted during the late 2006/early 2007 monitoring event that many of the recovering sponges had multiple barrels. Notes were recorded for 92 recovering sponges, and 59 were identified with one barrel, 14 had two barrels, and 19 had three or more barrels (Fig. 5).

Table 2: Number of sponges tagged within each monitoring zone (MZ) in 2003, and the number of sponges recovering, missing, and percent recovery from the late 2006/early 2007 monitoring event.

MZ	Total Tagged	Recov.	Missing	% Recov.
1	15	13	2	87
2	15	12	1	80
3	14	10	4	71
4	12	11	1	92
5	12	11	2	92
6	11	11	0	100
7	5	5	0	100
8	10	10	0	100
9	10	10	1	100
10	2	2	0	100
11	8	7	1	88
Total	114	102	12	90

Figure 5: Recovering barrel sponge with multiple barrels growing from the sheared base.

Discussion

The high latitude southeast Florida reef system is offshore a heavily populated coast and is therefore subject to multiple natural and anthropogenic stressors. Strong storm events, unauthorized injury events such as ship groundings, and authorized coastal construction projects potentially impact reef resources. There have been few efforts with almost no information recorded on restoring sponge populations and documenting recovery. Generally, reef restoration activities and recovery monitoring efforts concentrate on stony corals. Sponges are a dominant component of the southeast Florida reef system contributing greatly to benthic cover and density (Gilliam et al. 2007, 2008). Therefore, information on the capacity of sponges to recover and techniques to restore sponge populations following injury are needed.

Even though legal issues limited the information that may have been available, this study provides an example of the capacity of the giant barrel sponge, *Xestospongia muta*, to recover from severe injuries. Observed recovery of all sponges (notched and sheared) within several months of the injury event was over 93% and after four years was nearly 90% (Tables 1 - 2).

These recovery rates are higher, yet comparable to, those documented for *X. muta* offshore the Florida Keys, in which 30 out of 37 (81%) *X. muta* were considered recovered 13 months after suffering injury from a ship grounding (Schmahl 1999).

Although the growth rates of the recovering sponges in this study were estimates only and were highly variable, they fell within the range of other documented upward linear growth rates in the Florida Keys (Schmahl 1999, McMurray et al. 2008). Interestingly, mean injured sponge growth rates were higher in Broward County (2.78 cm y^{-1}) than recovering sponge growth rates in the Florida Keys (1.94 cm yr^{-1}, Schmahl 1999). Regardless of this difference, these elevated growth rates provide some evidence for the supposition that recovering sponges have higher-than-normal growth rates (1.85 ± 1.10 cm yr^{-1}, McMurray et al. [2008]).

Two methods were used to reattach sponge fragments. Although 401 total fragments were reattached, at the time the monitoring zones were established only 26 fragments were identified within the 11 zones. The exact fate of the remaining 375 fragments is unknown. They were either lost prior to zone establishment or were present within the injury areas but outside of the established zones. None of the six monitored fragments secured with cement were present in late 2006/early 2007. Observations immediately after cementing showed that the fragment was initially held to the substrate. It is likely

that due to the mortality of sponge tissue in contact with the cement, the fragment was not held in contact with the substrate for a time period necessary for the sponge tissue to grow. Five of 20 monitored fragments attached by the wire were found attached to the substrate in late 2006/early 2007. For those five the wire was able to hold the fragments tight against the substrate long enough for sponge tissue to grow. Growth was also observed over the wire. This limited data suggests that if *X. muta* fragments can be secured tightly against the reef substrate for a sufficient length of time, they are capable of forming a new attachment and will continue to grow. Because of the small sample size we can not conclude the attachment of *X. muta* fragments using wire is the best method and other methods should be evaluated. For some of the fragments, wave and surge conditions created a "sawing" effect with the wire, where it sliced downward into sponge tissue. Subsequently, the fragment was unable to remain in contact with the substrate, thereby preventing the stability required for reattachment. It is also interesting to note that several fragments that were not placed in their original growth orientation were still able to successfully reattach. These fragments were essentially "half barrels" and the portion of the barrel top, which was initially vertical, was attached horizontally (Fig. 4).

This study was interrupted for a period of three years due to legal issues associated with the injury event. This gap resulted in a significant amount of data loss especially in terms of sponge size class associated rates of new growth and causes of mortality. Nonetheless, this limited study does provide important information on the recovery of *X. muta* offshore southeast Florida, USA and supports the necessity for comprehensive, long-term monitoring efforts. Current research includes a controlled study to further explore *X. muta* recovery, growth, causes of mortality, and effectiveness of reattachment methods.

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A study of the effect of sediment accumulation on the settlement of coral larvae using conditioned tiles

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Abstract. Studies have shown that marine biofilms play an important role in coral larval settlement. Biofilms are formed when substrata are immersed in seawater over a period of time in a process known as conditioning. This study investigated the settlement of *Pocillopora damicornis* larvae on concrete tiles which had been conditioned in running seawater for one, two, four, six and eight weeks, with unconditioned tiles used as controls. Results showed that larval settlement appeared to be higher on unconditioned tiles. Two-way ANOVA indicated no significant differences in larval settlement densities in treatments and controls, but significant differences in the choice of settlement surfaces preferred. Larvae preferred to settle on the sides of the tiles, compared to the top and bottom surfaces. Regression analysis conducted on the weight of sediment accumulated and the density of settled larvae indicated a negative and significant correlation between the two variables. Sediment accumulation on biofilm layers growing on underwater substrata likely affects the suitability of these surfaces for larval settlement. This factor must be taken into account when designing artificial surfaces to enhance coral larval settlement for reef rehabilitation.

Key words: *Pocillopora damicornis*; larval settlement; biofilm; sediment

Introduction

The life history of sessile marine invertebrates is affected by the behaviour of larvae during the planktonic and settlement phases as well as by post-settlement mortality (Keough and Downes 1982). Various environmental, chemical and biological cues influence larval settlement. Crustose coralline algae (CCA), skeleton and coral rubble have been used to provide the necessary cues to induce settlement of corals (Morse et al. 1988; Heyward and Negri 1999; Webster et al. 2004). Biological cues such as marine biofilms also play an important role in coral larval settlement.

Marine biofilm formation occurs on any substrate immersed in natural seawater (Wimpenny 1996). A layer of organic molecules is immediately formed on the clean substrate surface when placed in seawater. During conditioning, marine bacteria, diatoms and algal spores grow on the surface, and sediment also accumulates on the surface to form a complex biofilm layer (Kirchman et al. 1982; Mitchell and Maki 1988; Gilmour 1999). The formation of marine biofilms is a dynamic colonization process that may change over time (Webster et al. 2004; Shikuma and Hadfield 2006). The settlement of larvae is affected by the length of time a natural marine biofilm has developed on a substrate. The layer of marine biofilm potentially affects the recruitment of larval benthic invertebrates (Characklis and Cooksy 1983; Webster et al. 2004). An increase in the duration of substrate conditioning has been reported to significantly increase the rate of coral larval metamorphosis (Webster et al. 2004).

The absence of sediment on substrate is essential for coral settlement (Harrington et al. 2004). However, coral reefs are increasingly exposed to natural and anthropogenic disturbances in the past few decades (Rogers 1979; Wilkinson 1998). These disturbances include increased nutrient levels, sedimentation, pollution and eutrophication in the marine environment, and have been reported to promote succession changes in reef communities (Birkeland 1987; Belliveau and Paul 2002; Fabricius 2005). A study showed that suspended sediment, and the accumulation of sediment on the upper surface of settlement substrate negatively affected larval settlement of *Acropora digitifera* (Gilmour 1999).

Singapore is a highly urbanised country, and has a steadily expanding economy. The marine environment has contributed to Singapore's success as one of the world's busiest ports and one of the

largest oil-refining centres (Chou and Goh 1998). However, the presence of numerous human activities along the coastline, such as shipyards and coastal development (Chua and Chou 1992), has resulted in degradation of the reefs. High sedimentation (45 mg cm^{-1} day^{-1}) has been recorded in Singapore waters (Low and Chou 1994). Generally, coral reefs do not extend beyond the 6 m depth due to high water turbidity and sediment load (Chou and Tun 2005).

The objective of the present study was to investigate the effect of conditioning artificial tiles in seawater, on the settlement of *Pocillopora damicornis* larvae. The ease of collection and maintenance of *P. damicornis* larvae, in addition to the regular planulating cycle of the adults make this species of coral a suitable test organism for the present study. This study also examined the accumulation of particulate matter on biofilm layers formed on conditioned tiles, and their effects on larval settlement.

Materials and Methods

Preparation of settlement substrates and larval collection

Cement tiles (of dimensions: 10x10x1cm) containing 10% of coral rubble (10% CR) were used as settlement substrates. Tiles (placed horizontally) were conditioned in flow-through seawater systems in indoor aquaria. Tiles were conditioned for 1, 2, 4, 6 and 8 weeks, making up five treatments. All experiments were conducted at the Tropical Marine Science Institute under aquarium conditions. Adult colonies of *P. damicornis* were collected from Singapore reefs and maintained in aquaria for planulation. Planulation occurred after the new moon, and *P. damicornis* larvae were collected in overflow collectors.

Effect of duration of tile conditioning on coral larval settlement

The larval settlement experiments were carried out in March 2007. A single experimental tile was placed in a 2 L polyethylene tank containing UV-treated seawater and 20 larvae were introduced into each treatment tank. Four replicate tanks were prepared for each treatment and Controls, which comprised clean, unconditioned tiles. The numbers of coral larvae which had settled on each tile were enumerated after a 10-day exposure experimental period.

Determination of particulate matter

In a larval settlement experiment repeated in February 2008, a determination was also made of the amount of particulate matter that had settled on the experimental tiles.

After the end of the exposure experiment and enumeration of settled larvae, particulate matter that may have collected from the top surfaces of settlement tiles were washed off with gentle agitation in clean filtered UV-treated seawater. Particulate matter removed from individual tiles was filtered in the laboratory onto Whatman GF/C filter paper. The filter paper was then dried in an oven at 65ºC over 24h, and the dry weight of the filtered particulate matter obtained thereafter.

Statistical analysis

A log (x+1) transformation was applied to the data (density of settled larvae) before statistical treatment. Two-way Analysis of Variance (ANOVA) with Tukey's post-hoc test were conducted on the density of settled coral larvae to determine if significant differences in settlement existed in the different treatments (different tile conditioning durations), and if coral larvae had a preference for a tile surface (top, sides, or bottom). Linear regression analysis was performed on the dry weights of particulate matter and numbers of settled larvae to determine possible relationships.

Results

The 10% CR tiles conditioned in aquaria for periods from 1 to 8 weeks showed different degrees of biofilm formation (Fig. 1).

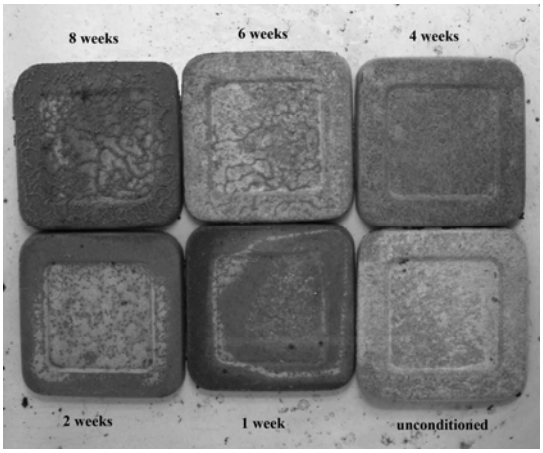

Figure 1: Surfaces of tiles conditioned for different periods of time in a running seawater system in indoor aquaria, showing different degrees of biofilm formation.

Two-way ANOVA on density of larval settlement data showed no significant interaction between the duration of tile conditioning and preferred settlement surfaces (top, sides, or bottom) on experimental tiles ($p > 0.05$; Table 1). Tukey's test revealed no significant differences in the mean settlement densities on tiles conditioned for different periods of

time ($p > 0.05$), but significant differences in settlement on different surfaces of the tiles ($p<0.05$, Table 1 and Fig. 2).

Table 1 Results of 2-way ANOVA on coral settlement density crossed with factors: duration of tile conditioning (0, 1, 2, 4, 6, and 8 weeks) and tile surfaces (top, sides or bottom), and results of Tukey's test. (* indicates significant treatment effects)

Factors	*df*	*F*	*p*
Duration of conditioning	5	1.923	>0.05
Tile surface	2	15.800	<0.05*
Duration × Surface	10	1.977	>0.05

The mean number of settled larvae showed a tendency to decrease with increasing periods of tile conditioning (Fig. 3). The highest settlement rate occurred on the clean, unconditioned tiles (Controls), and there was a gradual decrease in larval settlement on tiles conditioned for longer periods of time (Fig. 3). These results indicated that the larvae preferred to settle on clean, unconditioned 10% CR tiles.

The mean density of larvae that settled on the sides of the tiles were significantly greater than at the top surfaces of the tiles (Fig. 2).

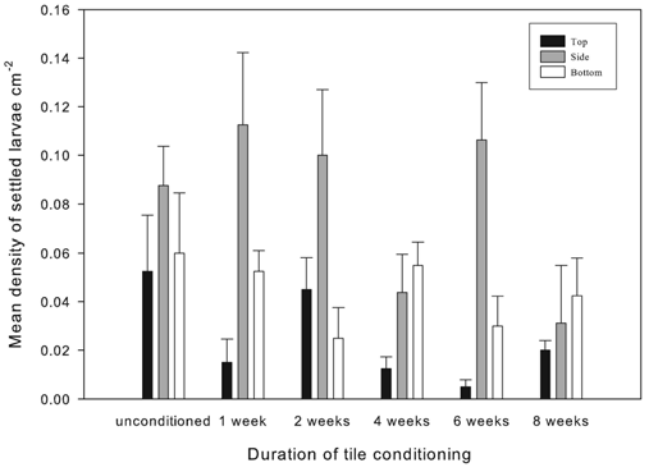

Figure 2: Mean density of settled *P. damicornis* larvae observed on different surfaces of tiles conditioned for different periods of time. (Means of 4 replicate aquaria; Error bars show standard error)

Regression analysis indicated a significant, negative relationship between the mean number of settled larvae and weight of particulate matter on experimental tiles ($r^2 = 0.512$, $p < 0.05$, $n = 24$; Fig. 4).

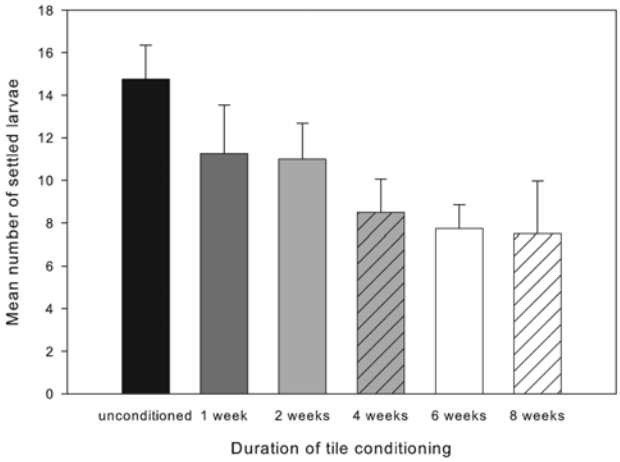

Figure 3: Mean number of settled *P. damicornis* larvae observed on 10% CR tiles conditioned for different lengths time over a 10-day experimental period in aquaria. (Means of 4 replicate aquaria; Error bars show standard error)

Discussion

P. damicornis larvae showed decreasing rates of settlement in relation to the duration of tile conditioning. In the experiment, larvae had a clear propensity to settle on clean tiles, suggesting that conditioning of 10% CR tiles was not necessarily a requirement for promoting larval settlement. A possible explanation of the results obtained may be that the longer duration of tile conditioning in a natural flow-through seawater tank also resulted in the accumulation of higher amounts of fine sediment on the top surfaces of the tiles.

In the present study, larvae seemed to have a marked preference for the sides (vertical surfaces) of the tiles for settlement. In a settlement orientation study of the corals, *Platygyra sinensis* and *Oxypora lacera*, larvae were significantly affected by physical and biological factors under natural conditions (Babcock and Mundy 1996). Settlement on vertical surfaces was also observed in an experiment which reported that tiles positioned between angles of 60° and 90° induced higher larval settlement (Carleton and Sammarco 1987).

Increasing sediment accumulation was observed on the top surfaces of tiles conditioned for longer periods of time in the study repeated in February 2008. The results also demonstrated that the presence of fine sediment decreased the effectiveness of potential suitable substrates for larval settlement of *P. damicornis* (Fig. 4). Sediment has been reported to inhibit the settlement of coral larvae (Hodgson 1990; Babcock and Davies 1991; Babcock and Mundy 1996). Larvae respond to negative chemical or physical cues due to the accumulation of sediment layers on the substrates (Hodgson 1990). Another

study demonstrated that suspended sediments and the accumulation of sediment on the upper surfaces significantly inhibited larval settlement of *Acropora digitifera* on CCA-covered substrates (Gilmour 1999).

It is likely that differences in microbial density and microbial community composition results from conditioning tiles for different durations of time. Few studies have focused on the role of the microbial biofilms on coral settlement. In tropical waters, the composition of marine biofilms fluctuate over time (Webster et al. 2004). Surfaces with different compositions, densities, ages and origins of biofilm attract different marine invertebrate larvae to settle (Parsons et al. 1993; Wieczorek and Todd 1998; Patel et al. 2003), and many larvae prefer to settle and metamorphose on multi-species biofilms (Kirchman et al. 1982; Patel et al. 2003). The community composition of the biofilm depends on the bacterial species which colonise the substrates. For example, *Pseudoalteromonas* bacteria isolated from CCA was able to induce larval settlement of *Acropora willisae* (Negri et al. 2001). Although the conditioned tiles may have developed a complex layer of microbial communities on their surfaces, this may have enhanced the accumulation of silt and sediment, rendering the surfaces unfavourable to the coral larvae. More studies are needed to investigate and to understand the role that microbial communities play in coral larval settlement.

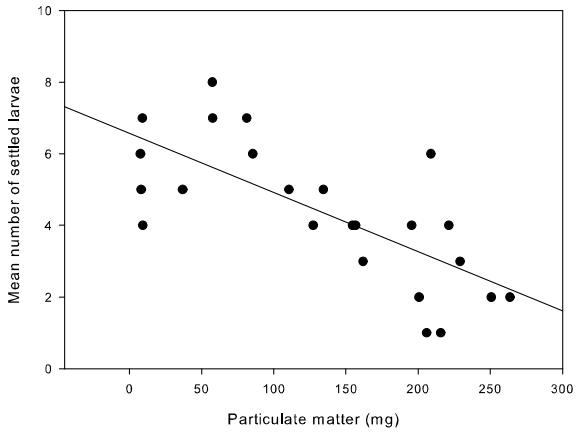

Figure 4: A negative relationship between mean number of settled larvae and the weight of particulate matter on conditioned tiles. The solid line through the data represents a least-squares regression.

Pocillopora damicornis is a brooder with a monthly planulation cycle. The larvae of coral species that are brooders tend to demonstrate more general responses to cues for metamorphosis and settlement (Baird and Morse 2004). It would be interesting to investigate the effects of substrate conditioning with sediment-stress on larvae from other species of corals which are broadcast spawners, to determine if similar responses are elicited.

In conclusion, increasing durations of substrate conditioning did not enhance larval settlement, possibly due to increasing accumulation of fine sediment on the substrates. Therefore, the characteristics and properties of substrates should be carefully considered in the selection of substrate surfaces to ensure high larval settlement. More research is necessary to investigate the physical and chemical properties of artificial substrates, the composition of biofilm/sediment layer found on biologically conditioned tiles, and species-specific preferences for coral larval settlement.

Acknowledgements

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Coral recruitment and community development: the Broward County artificial reef compared to adjacent hardbottom areas, five years post-deployment

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Abstract. Artificial reefs have been utilized as a tool for reef conservation, rehabilitation and mitigation. The success of artificial reefs is measured by their ability to mimic the natural hardbottom environments which they are intended to replace. Despite the numerous artificial reef studies, there are few comparative studies between artificial and natural reefs. This study examines recruitment and subsequent development of coral communities on an artificial reef, and compares them to adjacent natural hardbottom in Broward County, Florida, USA. We compare artificial to natural hardbottom coral communities by examining abundance, diversity and size class distribution over time. Scleractinian and octocoral abundance, diversity and average size on the artificial reef was nearly equal to those on the natural nearshore hardbottom five years post-deployment. This study presents the data from each of the seven monitoring events and discusses the variables contributing to coral recruitment and community composition on artificial reefs.

Key words: recruitment, artificial reefs, mitigation

Introduction

Coral reefs worldwide are in a state of decline and experiencing considerable degradation (Wilkinson 2000). Artificial reefs are frequently utilized as a means of rehabilitating and restoring degraded natural reefs (Bohnsack and Sutherland 1985; Seaman 2002), and in recent years artificial reefs have been deployed to prevent or mitigate for impacts resulting from anthropogenic damage (e.g., coastal development, ship groundings, pollution, etc.) (Spieler et al. 2001).

A majority of artificial reef studies have focused on the early colonization stages of benthic communities, while relatively few have included long-term monitoring to evaluate the development of artificial reef communities beyond the initial successional phases. In spite of the considerable attention paid to artificial reefs, there is also a scarcity of comparative studies between artificial and natural reef communities (Perkol-Finkel and Benayahu 2005). If we are to successfully utilize artificial reefs as tools for rehabilitation and restoration of degraded marine habitats, then more emphasis must be placed on evaluating the ability of benthic artificial reef communities to mimic those found on the natural reef (Perkol-Finkel and Benayahu 2007).

The current study presents results of five years of monitoring following the deployment of a limestone boulder artificial reef in Broward County, Florida, USA. In particular, the scleractinian and octocoral communities on the artificial reef are compared to those found on adjacent natural hardbottom substrate. Scleractinian and octocoral abundance, diversity and size class distribution are compared between the artificial and natural reef communities for each of seven monitoring events in order to evaluate the success of the artificial reef in resembling the natural community it was intended to replace.

Materials and Methods

Artificial and natural reef monitoring protocol

Construction of the Broward artificial reef was completed September 2003. The artificial reef was constructed as one layer of limestone boulders (1.2-1.8 m in maximum diameter) placed in the nearshore zone in approximately 4 to 6 meters mean water depths. Coral communities were evaluated using the Coastal Planning & Engineering, Inc. Benthic Environmental Assessment for Marginal Reefs (BEAMR) method (Lybolt and Baron 2006). A total of 54 30-meter long permanent transects were established on the artificial reef and the adjacent natural hardbottom (27 artificial transects, 27 natural transects). BEAMR was conducted along each transect using 12 replicate 1.0-m^2 quadrats (1.0 m x 1.0 m) spaced along the transect every 2.5 meters, starting at 0.0 m. Scleractinian coral colonies were identified to species level and octocoral colonies

were identified to genus level. Size was measured to the nearest cm. All natural and artificial transects were monitored 9-, 12-, 18-, 24-, and 36-months after artificial reef deployment. A subset of artificial reef transects was also sampled 48-months post-deployment. The artificial reef transects were all surveyed again at 60-months post-deployment.

Data analysis

Using PRIMER (v6) (Clarke and Gorley 2006), a Bray-Curtis similarity coefficient matrix was derived based on scleractinian and octocoral abundance per meter area of hardbottom for artificial and natural sites for each monitoring period. This similarity matrix was used to track the scleractinian and octocoral community changes at the artificial and natural habitats over time. Non-parametric multidimensional scaling (MDS) ordinations were created from the similarity matrices for both scleractinian and octocoral datasets. Simple agglomerative hierarchical clustering (Cluster) and similarity profiles (SIMPROF) were performed on both datasets to determine statistically significant differences in community structure over time.

Using PRIMER (v6) (Clarke and Gorley 2006), species richness (*S*), Shannon diversity index (*H'*), Simpson index (*1-λ'*), Pielou's evenness index (*J'*), and Margalef's index (*d*) were calculated for artificial and natural sites for scleractinian and octocoral communities over time.

Coral size class structures for artificial and natural sites were analyzed by calculating the scleractinian and octocoral size class abundance per meter area of hardbottom for each monitoring period. Size class profiles were compared to track changes in artificial and natural areas over time.

Results

Scleractinian community

Results from the MDS show that the scleractinian community at the natural hardbottom sites has remained stable over time, whereas, the scleractinian community on the artificial reef has gradually become more similar to the natural hardbottom community from 9-months to 60-months post-deployment (Fig. 1). Results of the SIMPROF suggest that the there have been three significant shifts in the scleractinian community on the artificial reef from 9-months to 60-months post-deployment, at which time the scleractinian community at the artificial reef has attained 70% similarity to the natural hardbottom community (Fig. 1).

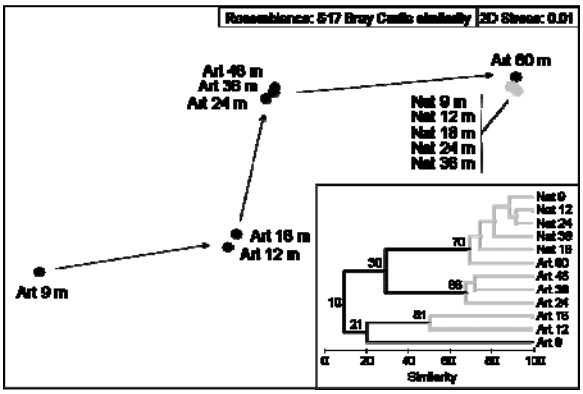

Figure 1: Two-dimensional MDS plot and corresponding SIMPROF dendrogram based on scleractinian abundance m^{-2} of hard substrate. Monitoring groups connected by grey lines cannot be significantly differentiated ($p \leq 0.05$).

Species richness at the natural sites remained between 11 and 14 species throughout monitoring (Table 1), whereas, species richness at the artificial reef climbed steadily throughout the duration of monitoring from 2 to 13. Diversity indices indicate that scleractinian diversity on the artificial reef increased steadily and by 60-months post-deployment, all four diversity indices surpass those of the natural reef (Table 1).

Table 1: Measures of scleractinian diversity on artificial reef and natural hardbottom.

	Scleractinians									
	Species Richness (S)		Shannon (H')		Simpson ($1-\lambda'$)		Pielou's (J')		Margalef's (d)	
Post-Deployment	Art	Nat	Art	Nat	Art	Nat	Art	Nat	Art	Nat
9-month	2	12	0.657	0.885	0.499	0.550	0.918	0.586	0.380	1.554
12-month	3	13	0.443	0.543	0.235	0.284	0.404	0.212	0.577	1.632
18-month	5	13	1.149	0.838	0.614	0.310	0.733	0.322	0.949	1.705
24-month	9	14	1.284	0.971	0.607	0.375	0.622	0.358	1.276	1.778
36-month	12	11	1.714	0.566	0.775	0.202	0.690	0.232	1.777	1.481
48-month	8	****	1.526	****	0.659	****	0.735	****	1.187	****
60-month	13	****	1.349	****	0.556	****	0.526	****	1.578	****

Scleractinian size class distribution at the natural hardbottom areas remained relatively unchanged from 9-months to 36-months post-deployment (Fig. 2). In general, the artificial reef scleractinian size class structure experienced steady increases in the three smallest size classes from 9-months to 48-months. Between 48-months and 60-months, the smallest size class (1-2 cm) experienced a five-fold increase and the 3-5 cm and 6-15 cm size classes experienced a three-fold increase, resulting in similar scleractinian size class structures at the artificial and natural habitats 60-months post deployment (Fig. 2).

Octocoral community

Results from the MDS show that the octocoral community at the natural hardbottom sites has remained stable overtime, whereas, the community on the artificial reef has gradually become more similar to the natural hardbottom community from 9-months to 48/60-months post-deployment (Fig. 3). Results from the SIMPROF indicate that by 48/60-

months post- deployment, the octocoral community on the artificial reef is 63% similar to that of the natural hardbottom sites (Fig. 3).

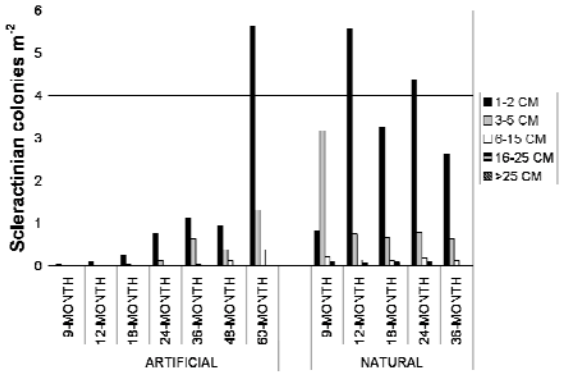

Figure 2: Scleractinian size class distribution on artificial reef and natural hardbottom for each monitoring period.

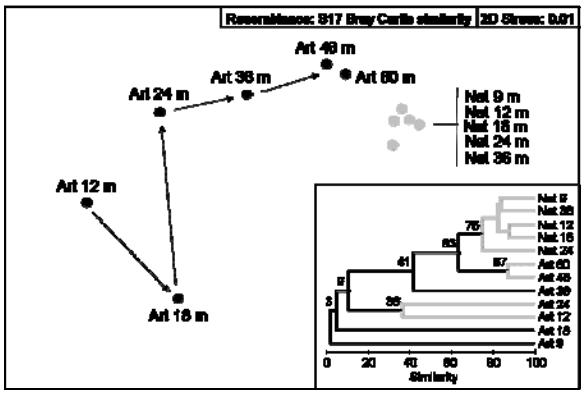

Figure 3: Two-dimensional MDS plot and corresponding SIMPROF dendrogram based on octocoral abundance m^{-2} of hard substrate. Monitoring groups connected by grey lines cannot be significantly differentiated ($p \leq 0.05$). Nine-month artificial reef monitoring event is not depicted in this figure, as the distance between it and all other subsequent events was too great to fit.

Species richness at the natural site remained stable throughout monitoring, between 8 and 11 (Table 2). Species richness at the artificial reef was 11 by the end of monitoring, equaling that of the natural sites. Diversity indices indicate that octocoral diversity on the artificial reef increased steadily, and by 60-months post-deployment resembled diversity observed on the natural reef (Table 2).

The artificial reef octocoral size class profile by 48/60-months post-deployment resembles that of the natural hardbottom, however, octocoral abundance was still greater at the natural sites than the artificial reef (Fig. 4). The abundance of octocoral recruits (1-5 cm) increased steadily throughout monitoring, while the level of recruitment on the natural hardbottom remained relatively constant.

Table 2: Measures of octocoral diversity on artificial reef and natural hardbottom.

	Octocorals									
	Species Richness (S)		Shannon (H')		Simpsons (1-λ)		Pielou's (J)		Margalef's (d)	
Post-Deployment	Art	Nat	Art	Nat	Art	Nat	Art	Nat	Art	Nat
9-month	****	8	****	1.999	****	0.757	****	0.917	****	1.573
12-month	3	10	0.952	1.886	0.668	0.832	0.736	0.824	0.714	1.372
18-month	2	11	0.451	1.936	0.321	0.824	0.650	0.803	0.455	1.518
24-month	8	11	1.591	2.169	0.701	0.888	0.862	0.904	1.359	1.582
36-month	8	8	1.895	1.999	0.808	0.765	0.828	0.947	1.468	1.284
48-month	7	****	1.922	****	0.881	****	0.877	****	1.027	****
60-month	11	****	1.969	****	0.792	****	0.851	****	1.648	****

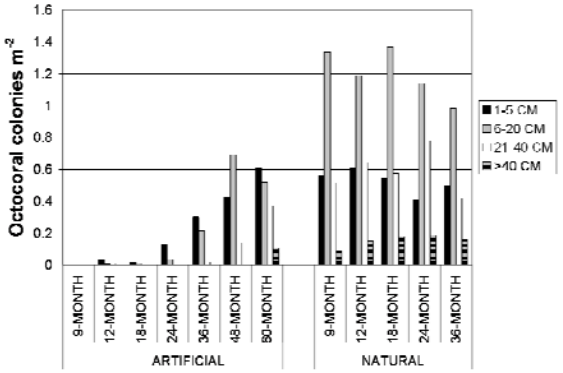

Figure 4: Octocoral size class distribution on artificial reef and natural hardbottom for each monitoring period.

Discussion

The Broward County artificial reef has succeeded in providing suitable habitat for recruitment and development of scleractinian and octocoral communities. These developing communities are becoming more similar in abundance, diversity and size class structure to the communities found on the adjacent natural hardbottom over time. As the MDS's suggest, by 60-months post-deployment, the scleractinian and octocoral communities on the artificial reef appear to be developing at slightly different rates, with the scleractinians and octocorals reaching 70% and 63% similarity, respectively, to the natural sites.

It is unclear why the scleractinian community appears to be more established, especially considering that only 5% of scleractinians on the natural hardbottom have reached reproductive size (>5 cm), while 30% of octocorals in the natural population are reproductive (>20 cm) (Bak and Engel 1979; Soong 1993; Gutiérrez-Rodríguez and Lasker 2004). It would be expected that this proportional difference in reproductive adults on the natural hardbottom would directly influence the level of recruitment onto the adjacent artificial reef. This reasoning seems to explain the observed trends until the last monitoring event: from 48-months to 60-months post-deployment, the scleractinian community at the artificial reef experienced a four-

fold increase in overall abundance and a five-fold increase in recruits (1-2 cm).

It is well understood that hurricanes or severe storms can reduce coral recruitment rates (Connell 1997); considering this in context of South Florida's active 2004 and 2005 hurricane seasons (corresponding to the 12-month and 24-month monitoring events) may explain the low recruitment levels leading up to 60-months post-deployment. A second explanation for the delay in high scleractinian recruitment levels could be related to time needed for organisms that produce chemical cues and substrate that enhance coral settlement to the artificial reef (e.g. crustose coralline algae) (Morse and Morse 1984; Vermeij and Sandin 2008).

Although this data shows that both the scleractinian and octocoral communities on the artificial reef are becoming increasingly similar to those on the natural hardbottom, structural differences between the low-relief natural nearshore habitat and the high-relief limestone boulders of the artificial reef are expected to lead to distinct climax communities (Baynes and Szmant 1989; Glasby and Connell 2001; Perkol-Finkel et al. 2006). The elevated surfaces of the limestone boulders will likely permit some species of corals to persist and grow that are unable to tolerate the ephemeral nature of the low-relief natural hardbottom. It is expected that the artificial reef will develop a climax community that will be more diverse than that on the natural hardbottom, and may include larger individuals and more colonies than found on natural hardbottom habitat. However, to date, the Broward artificial reef is succeeding as mitigation for natural hardbottom by harboring a coral community that continues to increase in resemblance to that of the adjacent natural hardbottom coral community.

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Development of a coral nursery program for the threatened coral *Acropora cervicornis* in Florida

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Abstract. The staghorn coral, *Acropora cervicornis*, was once a dominant reef-building species throughout the Caribbean region. Due to hurricanes, disease, and other human and natural stressors, its populations have declined dramatically in the last few decades. In an attempt to propagate this species for local restoration efforts, coral gardening was initiated in Florida, USA in 2007. Branches of *A. cervicornis* were clipped from donor adult colonies from Biscayne National Park and cemented onto cinder blocks in an underwater nursery. Eighty-eight fragments were monitored regularly during the first four months after transplantation to evaluate patterns of mortality attributable to the collection and transplant methods and to assess initial patterns of growth. Fragment mortality of 17.3% was documented for the first 8 weeks after transplantation, but decreased to <1% in subsequent monitoring intervals. Although there was no significant difference in growth rates between fragments glued in horizontal and vertical position, larger-sized fragments (> 5 cm) grew significantly faster than small (< 3 cm) and medium (3-5 cm) fragments.

Key words: coral gardening, coral nursery, *Acropora cervicornis*

Introduction

The scleractinian coral *Acropora cervicornis* was once a dominant building species in Caribbean coral reefs during the past 500,000 years (Jackson 1994). This species, however, has experienced drastic population declines (>95%) during the last three decades attributed to multiple factors that include increased sea-surface temperatures and associated bleaching, hurricanes, and white band disease (Bruckner and Hourigan 2002). The recent decline of *A. cervicornis* across its full range has prompted the listing of this species as 'threatened' under the U.S. Endangered Species Act in 2006. Furthermore, this species relies heavily on asexual reproduction via fragmentation and has limited success in sexual recruitment, demographic attributes that slow population recovery beyond local habitats (Bak and Engel 1979; Tunnicliffe 1981; Knowlton et al. 1990; Vargas-Angel et al. 2003). The local conservation of *A. cervicornis* populations as well as the build-up of stocks via active restoration can potentially improve recovery by enhancing rates of sexual recruitment (Vollmer and Palumbi 2007).

In the last decades, "coral gardening" (Rinkevich 1995, 2000, 2005; Bowden-Kerby 2001; Epstein et al. 2001; Shafir et al. 2006) has become an increasingly important tool in reef restoration. Coral gardening consists of growing corals in-situ at a nursery site, and then transplanting these coral fragments back onto natural reef environments once they have grown to an appropriate size (Rinkevich 1995, 2000; Epstein et al. 2001; Shafir et al. 2006). Coral gardening has been undertaken using varied methods of fragment or nubbin attachment such as mid-water wire frames, floating platforms, concrete, and suspended lines (Bowden-Kerby 2001; Shafir et al. 2006).

In this study, we describe the initial stages of a local attempt to enhance staghorn coral populations in Florida, USA. The gardening approach used in this study consisted of collecting fragments of *A. cervicornis* from adult colonies and growing these fragments in a coral nursery established within Biscayne National Park, Florida. Due to the depletion of *A. cervicornis* in Florida, the goal of this project is to collect minimal tissue from adult colonies (thereby limiting further depletion of adult stocks) and establish a methodology to maximize growth and survivorship of fragments to provide an expanding source of coral tissue for future restoration activities.

Here we report initial patterns of fragment survivorship and growth based on initial fragment size and orientation of attachment to transplant platforms. This study concentrates on the time period immediately following collection and transplantation of fragments to document directly the impacts of the collection, transportation, and cementation methods on fragment growth and survivorship. Based on the source of stress to the fragments collected, it was hypothesized that fragment mortality would be high initially but would decline with time as fragments

cemented themselves to the platforms and the regrowth process began. Initial high mortality followed by reduced mortality rates of surviving fragments were documented in other coral transplant experiments (Clark and Edwards 1995; Quinn and Kojis 2006).

Material and Methods

Staghorn coral nurseries in Florida

The nursery described in this study is part of a network of four staghorn nurseries established in the Florida Reef Tract from Broward County to the Middle Florida Keys using uniform methods (Nedimeyer and Johnson, unpublished). After a period of growth (6 mo – 1 yr) at the nurseries, the fragments will be transplanted to different habitats where staghorn corals were once abundant as well as habitats damaged by ship groundings.

Coral fragments

Donor adult *A. cervicornis* colonies were located at 11 different sites within Biscayne National Park. Coral fragments were carefully collected from the donor colonies using pruning scissors (Fig. 1).

The nursery

The nursery is located at 6 m depth in Biscayne National Park, Florida, US (25º 21.753′ N, 80º 9.985′ W). A sand patch adjacent to a reef was used to deploy a matrix of 30 cinder blocks. Each block contained a maximum of 10 vertical cement cylinders with a cement "puck" attached at the top (Fig. 1). The fragments were mounted to the puck using underwater epoxy. Identification codes were marked on the pucks using a stone engraver, black marker, and a coat of fiberglass resin. The cement pucks were designed so that the whole unit can be transplanted back to the various reef habitats after a period of regrowth without the need to handle the colonies. The pucks themselves will be cemented to the reef bottom at the future transplant sites.

In this study, we report growth data for 88 fragments that were measured regularly during the initial 4 months after transplantation (June to October 2007). Fragments were divided into 3 size classes: small (<3 cm in max length), medium (3-5 cm), and large (>5 cm). Within each size class, fragments were positioned either horizontally or vertically on the pucks (Fig. 2). At each survey, the cinder blocks were scrubbed clean with wire brushes to limit algal overgrowth and sediment accumulation.

Figure 1: Photographs depicting the steps in the coral gardening approach used in this project. 1) Adult *A. cervicornis* colonies. 2) Fragmented colony. 3) Fragment from which smaller sections were cut. 4) Nursery platform with transplanted fragments.

Monitoring and maintenance

Numerous studies have correlated coral growth and survivorship with colony size (e.g., Hughes 1984). For branching corals with complex morphology, it is often impossible to estimate whole-colony size (and growth) in the field and researchers have used the growth of a subset of branches as a proxy of whole-colony growth. In this study, the fragments used had a simple initial morphology (a single cylindrical branch) or simple branching morphology (maximum of 4 branches by the end of the study period). Thus, it was possible to measure and report the initial size and growth of fragments by calculating the sum of all branch lengths. This approach was used successfully to assess growth of *A. cervicornis* fragments and colonies by Bowden-Kerby (2001) and Quinn and Kojis (2006). As colonies grow in complexity, growth patterns will likely need to be assessed by measuring the average linear extension of a subset of marked branches. Patterns of linear growth were compared among fragments with a two-way ANOVA with size and orientation as main factors.

Results

A fragment mortality of 17.3% was documented in the first 8 weeks after transplantation. However, a large portion of this mortality (9.2%) was recorded during the first 3 weeks after transplantation. Mortality decreased to <1% of the remaining fragments during subsequent surveys (Fig. 3). No significant differences in the mean size of the surviving (4.4 cm ± 2.1) and dead fragments (4.1 cm ± 1.3) were observed during the first 8-week period ($p > 0.05$, *t* -test).

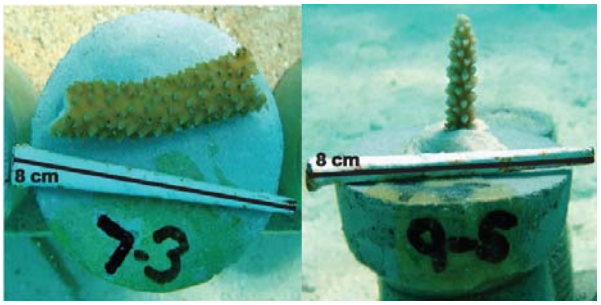

Figure 2: Photographs of staghorn corals cemented in horizontal and vertical growth position.

Fragment growth during the first four months after transplantation was influenced by initial fragment size. There was a significant difference ($p < 0.05$) amongst the linear extension rates of the 3 size classes, with the larger size class growing faster than the smaller ones (Fig. 4). Although fragments placed in horizontal position generally grew faster that those placed in vertical position (except for the smallest fragments), these differences were not significant. Lastly, no significant interaction was found between fragment size and orientation ($p > 0.05$).

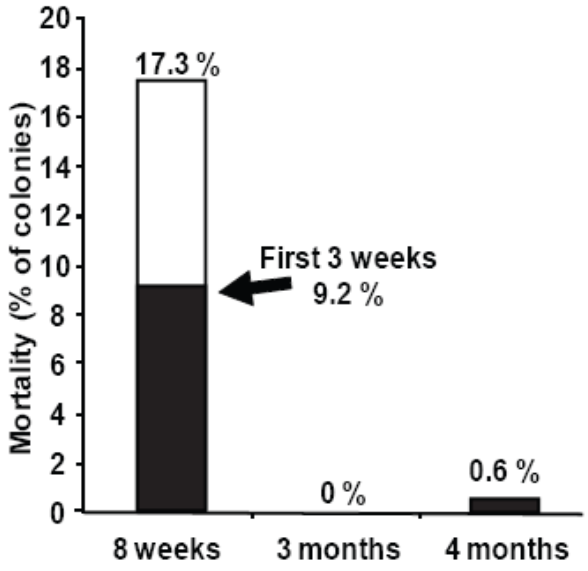

Figure 3: Mortality of fragments transplanted onto the coral nursery.

Discussion

The initial stages of the coral gardening program implemented for *A. cervicornis* in Biscayne National Park have been encouraging and this approach has the potential to become an important tool to provide an expanding stock for future local reef restoration efforts. Moreover, the combination of relatively low initial mortality and fast growth rates of fragments demonstrates that staghorn corals could be appropriate for a coral gardening program in Florida.

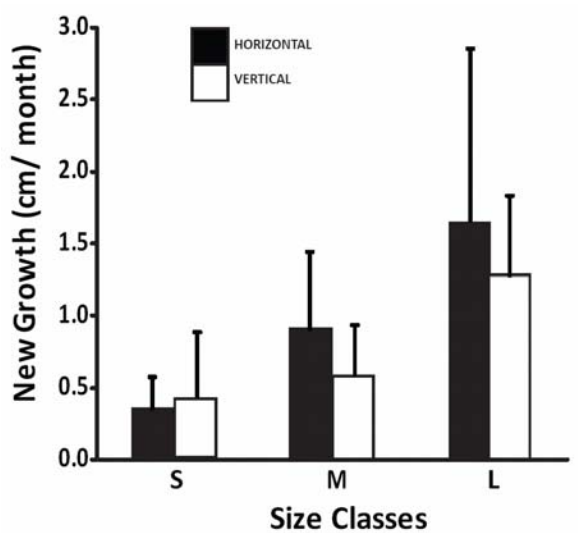

Figure 4: Mean growth (± SD) of transplanted staghorn fragments based on initial size and orientation.

Previous attempts to establish nurseries with staghorn corals provided initial fragment mortality estimates of 22% and 27% (average of 4 sites, recorded 6 and 11 weeks after fragmentation respectively) (Quinn and Kojis 2006). These mortality estimates are comparable to that in our nursery (17.3% after 8 weeks).

In the absence of major disturbances, the highest levels of mortality are commonly recorded during the first observation period following fragmentation, with mortality rates decreasing over time as fragments attach to the substrate and grow (Clark and Edwards 1995; Bowden-Kerby 2001). In agreement with these studies, the largest proportion of the mortality recorded took place within the first few weeks after transplantation (9.2% of fragments died within the first three weeks), and mortality rates decreased thereafter, indicating that the environmental conditions at the nursery site are adequate for fragment survivorship and growth. Likely factors influencing the early mortality patterns observed include stress due to fragmentation and high-temperature impacts. Initial fragmentation for this nursery was done during June and July of 2007, when sea surface temperatures were high (29–30ºC). Based on these observations, we concluded that, when possible, the fragmentation step of coral gardening should be performed when most other extraneous stressors, like high temperature, are minimized. .

The size of fragments is commonly associated with rates of survivorship and growth (Lirman 2000). In this study, fragment mortality was not associated with fragment size (for the range of sizes collected) but larger fragments exhibited faster initial growth rates compared to smaller fragments. This information is

important to determine the minimum size of fragments to collect for a gardening program. The faster growth rates of the horizontal fragments were unexpected because more living tissue from the bottom portion of these fragments was lost initially due to the contact with the epoxy and the cement base. However, fragments placed in a horizontal position have at least two terminal ends for potential new growth and branch development. By being able to extend from the additional growing end (compared to vertical fragments), horizontal fragments grew initially faster than vertical fragments. The continued monitoring of fragment growth will determine whether the initial faster growth rates of horizontal fragments is maintained over time as fragments develop complex branching patterns.

Maximizing growth rates of transplanted corals is a key goal of gardening programs. In this study, the maintenance conducted to remove sediments, macroalgae, and other coral competitors provided enhanced growth conditions for fragments. Under these conditions, the growth rates of staghorn fragments measured in the nursery compared favorably with the linear extension of adult staghorn colonies (10-15 cm/year) reported previously (Gladfelter 1984), indicating that even small fragments are capable of rapid growth rates when potential sources of competition and stress are removed periodically.

The propagation of *Acropora cervicornis* via a coral gardening approach implemented within in-water nurseries using low-cost materials (e.g., cement, epoxy, cinder blocks), can provide an effective method to expand declining stocks of the threatened staghorn coral in Florida. The results that help classify this new program as successful in its early stages include: (1) limited initial fragment mortality (< 20% of fragments were lost in 2 months following fragmentation); (2) rapid decline in subsequent mortality (< 1% between 2 and 4 months); and (3) rapid growth of even the smallest of fragments. The ultimate success of these restoration efforts will depend on the continued growth of fragments as well as the viability of nursery-reared colonies once transplanted back onto natural reef sites.

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Coral relocation for impact mitigation in Northern Qatar

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Abstract. A large-scale, long-distance coral relocation project was conducted as mitigation for impacts to hard coral habitat associated with marine construction activities offshore Ras Laffan Industrial City, Qatar. Working under the guidance of the Supreme Council for the Environment and Natural Reserves, Qatargas Operating Company Limited and its Venture partners engaged in a hard coral relocation project. The project salvaged and relocated over 4,500 hard corals from pipeline corridors to mitigate for pipeline installation impacts. Proven techniques for coral reattachment and newly developed methods to enhance coral survival were used for the mass recovery, transport, and reattachment of the corals. Corals were transported 46 km, a single day transit, from the north coast of Qatar to a coral habitat along the east coast and reattached with concrete. Initial monitoring of approximately 5% of the reattached corals and randomly selected reference corals indicates high survival rates. This project represents an option for off-site mitigation and is an example of proactive environmental regulation, corporate responsibility, and advanced field technology applied in concert to reduce impacts to a viable hard coral habitat.

Key words: Off-site mitigation, Coral relocation

Introduction

Qatargas Operating Company Limited (QG) and its Expansion Projects (i.e., Qatar Liquefied Gas Company Limited [II], Qatargas 3 and Qatargas 4 Joint Asset Development Team [QG3&4], and Common Condensate Single Point Mooring [SPM] Project) are installing five export pipelines within three corridors offshore the coast of Ras Laffan Industrial City (RLC), Qatar to expand their production capabilities in the Arabian Gulf.

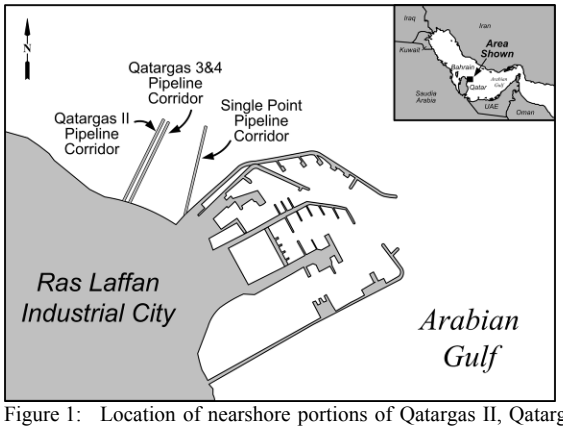

Figure 1: Location of nearshore portions of Qatargas II, Qatargas 3&4, and Common Condensate Single Point Mooring (SPM) pipeline corridors relative to Ras Laffan Port.

The pipelines will be installed on the seafloor from the offshore platforms to a point 2.5 km offshore RLC (Fig. 1). From 2.5 km offshore to the pipeline landing, the pipelines will be buried in three 7-m wide trenches. It was estimated that pipeline burial activities would affect a 50-m wide area along the 2.5-km distance for each pipeline corridor.

The Arabian Gulf, a semi-enclosed shallow sea, supports many complex and unique hard coral habitats. The Gulf is connected to the Indian Ocean by the Straits of Hormuz and experiences some of the widest seasonal fluctuations in water temperature and salinity recorded anywhere in the world. Gulf corals have adapted to fluctuations in temperature ranging from 10°C to 40°C and salinity changes from 28 to 60 ppt (Pilcher et al. 2000). Therefore, many endemic species are found in the Arabian Gulf but overall the coral habitats have low biological diversity. Recent bleaching events attributed to extreme high water temperatures occurred in 1996, 1998 and 2002 and reduced coral cover to less than 1% in many of the shallow water areas (Rezai et al. 2004).

The slow growing hard coral populations, which have only begun to recover from these bleaching events, are also threatened by anthropogenic impacts. Corals in sensitive coastal habitats are affected by turbidity and suspended sediments from land reclamation and dredging projects. Cooling water discharged from desalination and power plants exposes coral habitat to heated effluent water, generally 10°C above ambient temperatures, and toxins including anti-fouling and anti-scaling

chemicals. Nearshore construction projects for the oil and gas industry also threaten sensitive coral reef habitats.

The proposed new pipeline routes for Qatargas Expansion Projects cross sensitive coral habitats offshore RLC. The proposed pipeline corridors were surveyed in 2004 and 2006 to characterize habitats that would be affected by pipeline installation activities. The nearshore portions (defined as approximately 1.2 to 2.5 km offshore) of each of the pipeline corridors were characterized by low-relief hard bottom substrate and a biological community that includes hard corals at an average density of 0.47 colonies m^{-2} (Continental Shelf Associates Inc. 2005a, b, 2006a).

Based on the habitat characterization surveys, a Coral Management Plan (CMP) was prepared, approved by the Supreme Council for the Environment and Natural Reserves (now the Ministry of Environment), and implemented to mitigate the unavoidable impacts of pipeline installation activities to this sensitive habitat. The CMP recommended the reduction of biological impacts by salvaging and transplanting hard corals affected by pipeline installation rather than the alternative of compensating for destroyed corals by creating artificial reef habitats. Based on the observed average live hard coral density and coral relocation experience from other areas, approximately 4,500 hard corals were relocated from the area to be dredged to a fasht (shallow, emergent rock marine outcrop) coral habitat along the east coast of Qatar (Continental Shelf Associates Inc. 2006b).

Materials and Methods

Reattachment area

Water depth measurements, temperature and salinity water column profiles, substrate classifications, general habitat observations, and video and still photographs were collected at eight sites along the east coast of Qatar to determine the most suitable site for coral reattachment activities. Site 7 (25°33' 02.00"N, 051°37'22.50"E), known locally as the Fasht al Hurabi, was selected as the most suitable site for reattachment activities. The Fasht al Hurabi area is a crescent-shaped, 1–5 m deep, gently-sloping rock outcrop with strong north-to-south running currents. The coral community was historically diverse and thriving, and suffered mass mortality in the last decade likely due to elevated seawater temperature anomalies that occurred in the region. Since that time, recovery of this community has been slow, as reflected by the lack of acroporid branching corals (*Acropora* spp.) and the dominance of available hard substrate by opportunistic massive coral species (*Cyphastrea microphthalma* and *Porites harrisoni*) and associated biota. Coral skeletal remains indicate a once flourishing *Acropora* dominated coral community in shallow water at depths of 2–3 m, transitioning to a more massive coral species dominated community comprising the genera *Porites* and *Platygyra* at depths of 3–5 m.

Coral removal

A total of 4,517 coral colonies was chosen for removal based on a general set of criteria, i.e., selected colonies must be 1) representative of the species and densities present within the pipeline corridors; 2) greater than or equal to 10 cm in diameter; 3) preferably mounding and boulder growth forms rather than encrusting forms; and 4) relatively healthy with no obvious disease, bleaching, or partial mortality. Selected colonies were removed by shearing the coral/substrate attachment point using a hammer and masonry cold chisel and lifting the colony off in one piece, to the extent possible. Each coral colony was carried by hand or in a bucket to a 2 m x 2 m metal transport tray, where it was cached *in situ* in a single layer until all corals were detached and ready for transport to the relocation site. Once all corals had been cached, the transport trays were prepared for recovery by attaching a buoyed lifting bridle and small meshed covering to each of the transport trays.

Coral transport

A 33.5-m landing craft was used to transport the corals a distance of 46 km from the removal area to the relocation area. Approximately 2,250 corals were transported per transit to the relocation area. To maintain corals in ambient temperature seawater and a dark environment during the day-long transport, a large (44-m^2) covered circulating seawater pool was constructed on the deck of the transport vessel (Fig. 2). A large crane was used to bring cached coral colonies to the surface in the transport trays and place them in the pool on board the transport vessel. The pool was covered during transit to the relocation area to prevent or reduce stress to the corals from overheating and excessive solar illumination. Once at the relocation area, the filled transport trays were removed from the pool and lowered to the seafloor with the large crane. Once the transport trays were stable on the seafloor, transport bridles and nets were removed by scientific divers. Global positioning system (GPS) coordinates were recorded for each on-bottom transport tray cache location.

Coral reattachment

Corals were reattached with specialized concrete at 22 sites within the reattachment area (Fig. 3). Prior to attaching corals, the attachment surfaces of both the

coral and the selected receiver site were prepared by removing loose surficial debris and biota (i.e., algae and fouling organisms) to ensure proper bonding with the concrete. Manageable amounts of sand and cement were mixed in plastic buckets on the deck of support boats and then lowered to divers who placed sufficient amounts of concrete directly on the pre-cleaned substrate. The non-living base of the detached coral colony was then pressed firmly into the concrete mixture. Attached corals were checked periodically during reattachment operations to ensure their stability, address the aesthetic quality of the reattachment matrix, and dissipate cement residue that may have settled on adjacent living coral tissue and biota.

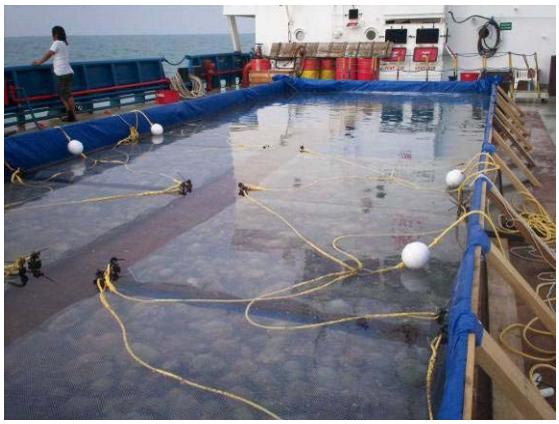

Figure 2: Loaded transport trays within the circulating seawater pool on the deck of the transport vessel.

Monitoring

Six of the 22 reattachment sites were randomly selected to bi-annually monitor the success of the coral relocation project and to assess and record detectable changes in the benthic community structure (Fig. 3). A subset of the relocated corals (approximately 5%) and natural (reference) corals were randomly selected, uniquely tagged, and mapped to monitor the relative coral health and success of the reattachment activities (Fig. 4). *In situ* observations of colony health and status of the cement bond as well as scaled digital photographs were collected for each monitored colony during all monitoring surveys. Four permanent 10-m transects were established at each of the monitoring sites to quantitatively document temporal changes in seafloor habitats and the associated benthic community. Temperature data loggers were installed to continually record seawater temperatures between monitoring surveys.

Results and Discussion

Hard coral colonies within the pipeline corridors generally were less than 30 cm in diameter with mounding or boulder morphologies that enabled the removal and reattachment of intact colonies. Relocated colonies ranged in diameter from 10 to 35 cm from seven species with mounding or boulder morphologies. Fragmentation of colonies was avoided because branching colonies were not encountered and the colonies were relatively small and manageable. Additionally, colonies were not fragmented as transplantation of large fragments or whole colonies has a greater likelihood of success compared to small fragments (<10 cm) (Plucer-Rosario and Randall 1987; Harriott and Fisk 1988; Edwards and Gomez 2007).

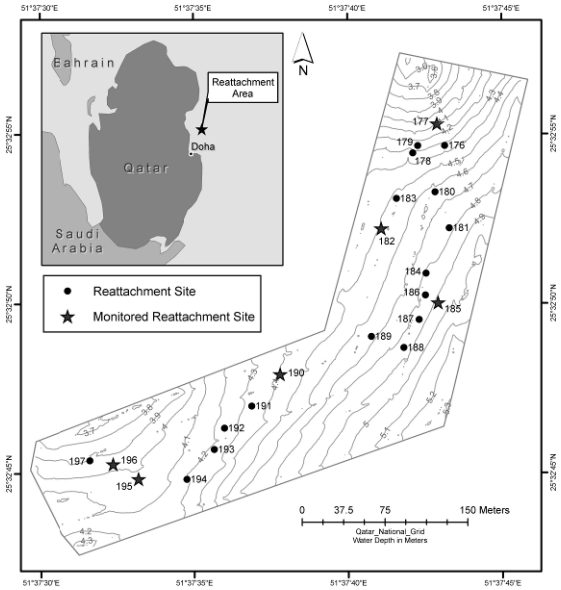

Figure 3: Coral reattachment and monitoring sites relative to the bathymetric contours at Fasht al Hurabi.

Figure 4: Monitored reattached colony of *Platygyra lamellina*.

Reattachment activities were completed in February 2007 with 4,548 colonies (4,517 transported colonies and 31 additional colonies found loose at the reattachment area) from seven species reattached at 22 sites at Fasht al Hurabi (Fig. 3).

The attachment status and relative health of 285 reattached colonies and 101 natural (reference) colonies was monitored bi-annually to assess the success of the relocation project (Table 1). After one year, 99% of the reattached monitored colonies survived compared to 79% survival of reference colonies, and a greater percentage of reattached colonies were healthy (58%) when compared to reference colonies (33%) (Fig. 4). The greater percentage of dead and unhealthy reference colonies compared to reattached colonies may be due to smothering from increased sedimentation observed during the 12-months post-reattachment survey since natural colonies are often located near the bases and sides of rocks. Coral colony health declined during the period between the 6-month and 12-month post-reattachment surveys. The decline was observed in both reattached and reference corals, which may be attributed to increased stress during the winter months due to extreme sustained low sea water temperatures documented by the on-site temperature data. Low temperature events and adverse effects on corals have previously been documented in the Arabian Gulf (Coles and Fadlallah 1991). Additional stressors experienced across the reattachment area during the month of February included increased sedimentation and a seasonal bloom of the brown alga *Colpomenia sinuosa* (Coles 1988).

Table 1: Summary of monitored coral colonies by species.

Scientific Name	Reattached	Reference
Anomastraea irregularis	10	1
Cyphastrea microphthalma	4	51
Favia sp.	123	8
Platygyra lamellina	46	12
Plesiastrea versipora	45	4
Porites harrisoni	55	18
Pseudosiderastrea tayami	2	7
Total	285	101

The benthic community was dominated by turf algae during the summer 2007 survey (6 months post-reattachment) and by *Colpomenia sinuosa* during the winter 2008 survey (12 months post-reattachment). Hard corals and other fauna (e.g., sponges and oysters) combined provided less than 12% cover during both surveys. Average hard coral density was low at all sites, ranging from 1.8 to 2.5 colonies/m^2, a slight decrease from 6 months post-reattachment (2.3 to 2.7 colonies/m^2).

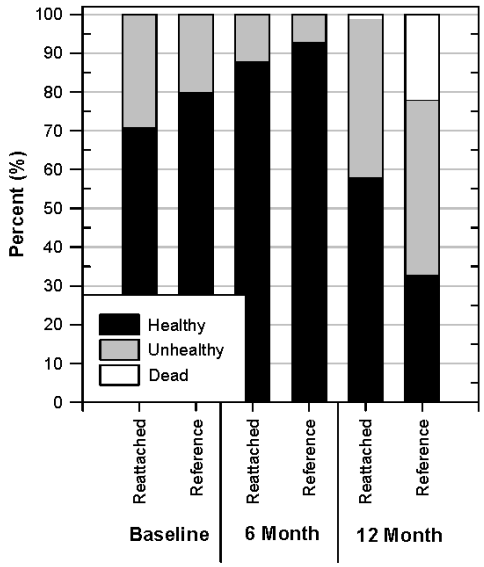

Figure 4: Comparison of the percent of healthy, unhealthy, and dead monitored colonies among monitoring surveys.

Seawater temperatures were similar at all of the monitoring sites, ranging from a high of 36.1°C in August 2007 to a low of 14.4°C in January and February 2008 (Fig. 5), representing a temperature range of 21.7°C. This range in water temperature is similar to the extreme temperature range previously documented on coral reefs in Qatar (Shinn, 1976) and is one of the greatest temperature ranges recorded on high latitude coral reefs. Minimum water temperatures fell below 18°C for 4 consecutive days in December (28 to 31 December 2007) and for 37 consecutive days beginning 7 January 2008 until 13 February 2008. During the latter extreme low water-temperature event, colonies were exposed to water temperatures below 16°C for 8 consecutive days in January (15 to 22) and again in February (1 to 8).

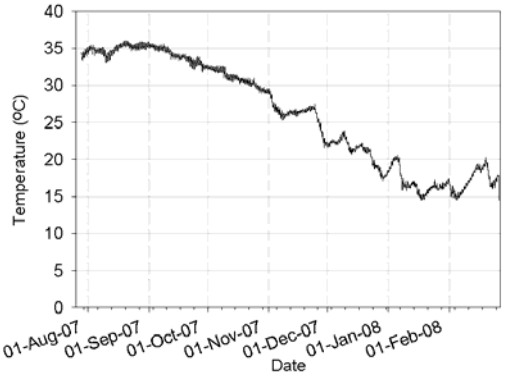

Figure 5: Continual seawater temperature data recorded from 27 July 2007 to 26 February 2008.

Conclusions

The results of this project indicate that hard coral relocation is a viable means of off-site mitigation for unavoidable impacts to sensitive coral habitats. After 1 year, 99% of monitored reattached colonies have survived. Although previous monitoring studies (Yap et al. 1992; Yap 2004) indicate that 1 year is an adequate period of time to evaluate the response of reattached corals to environmental conditions, biannual monitoring will be continued for at least two years following the transplantation to further assess the success of the relocation effort.

This successful project provides a benchmark for off-site mitigation that environmental regulators may utilize as a viable option for minimizing direct impacts to hard corals from offshore construction activities. Coral relocation in Qatar has been used as mitigation on another construction program based on the relative success of this project (CSA International Inc. 2008). Reattachment sites for future relocation activities should be carefully selected with similar environmental conditions (depth, temperature, salinity, turbidity, and benthic habitat type) to those at the removal site to increase the likelihood of coral survival. If possible, future relocation projects should consider conducting relocation activities during mean water temperatures to minimize stressful environmental conditions immediately after reattachment.

Acknowledgements

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The "Eco-Block" as a coral-friendly contrivance in port construction

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Abstract. Following observations of natural recruitment of coral colonies to breakwaters in Naha Port, Okinawa Prefecture in 1989, we launched monitoring surveys to explore the processes of coral settlement and growth on concrete armor blocks, along with the development of technology to enhance coral settlement and growth on such structures. The development concept centered on creating rough surfaces on conventionally smooth block surfaces, thus imitating the natural rugosity of reef substrates. In 1999, the findings from these surveys and experiments were then applied to the project "Eco-block", a wave-dissipating block with unevenly processed surfaces to enhance coral recruitment. Since the installation of Eco-blocks, the growth and recruitment of corals on the blocks has been recorded yearly. Eco-blocks with surface abrasions at about 5mm and 10mm in depth and width had higher coral settlement compared to unprocessed blocks.

Key words: coral restoration, port development, artificial structure, surface processing, increased settlement

Introduction

Coral communities are widely recognized for the crucial roles they play in subtropical and tropical coastal ecosystems (Costanza et al. 1997; Moberg et al. 1999). Among the world's coral reef seas, the southern area of Japanese waters surrounding the islands of Okinawa Prefecture has been described as a biological hot spot, an area with high diversity and a multitude of rare species (Roberts et al. 2002). Coral communities found in this area were extensively damaged following a severe bleaching in 1998, which was induced as a result of abnormally high sea temperatures (Hoegh-Guldberg 2000). These corals are now showing signs of recovery; yet, it is crucial to gain an insight into the coral growth and settlement before and after the bleaching event, as well as the development of restoration technologies to facilitate the recovery of corals from bleaching.

The Okinawa General Bureau, the Cabinet Office of Japan, also spare no efforts in promoting port development, in which port or harbor modification should follow "coral friendly" approaches. In 1989, coral colonies were found settled on wave-dissipating blocks of Naha First Breakwater in Naha Port. Every year since 1990, the settlement and growth of corals have been monitored annually on 30 fix quadrates. The results compiled until 2003 were reported by Ooka et al. (2006). In addition, focusing on this phenomenon as a way to make port construction more coral-friendly, we began the development of technology to enhance coral settlement and growth on concrete armor blocks. The development concept centered on creating roughness on conventionally smooth block surfaces, thus imitating natural substrates as near as possible. The initial experiment began in Naha Port in 1990, followed by the second one in 1991. The findings from these experiments were then applied in a project utilizing the "Eco-block" in 1999, a wave-dissipating block with abrasive protrusions on its surface. Since the installation of Eco-blocks, the growth and settlement of corals have been monitored yearly in 36 fixed quadrates placed on them. In this report, we analyzed the findings from these surveys and evaluated the effectiveness of such uneven processing on block surfaces in enhancing coral settlement.

Methods

Monitoring of coral settlement and growth: wave-dissipating blocks without surface processing

The purpose of this monitoring is to explore the coral settlement and growth on the unprocessed, smooth surfaces of artificial substrates. The surveys were conducted around Naha First Breakwater in Naha Port,

Japan. Live coral coverage and number of colonies were monitored annually over eight years between 1990 and 1997 at six different water depths C.D.L. (Chart Datum Level) -1, -3, -5, -7, -9 and -12m). The data were collected from three stations set up at each depth, and year-on-year changes were observed and reported (Yoshimi et al, 1998). The monitoring was then resumed in 2000, in response to the coral degradation caused by the bleaching in 1998, an extensive bleaching associated with unprecedented high sea temperatures around the world. The surveys were conducted on the same stations until 2007.

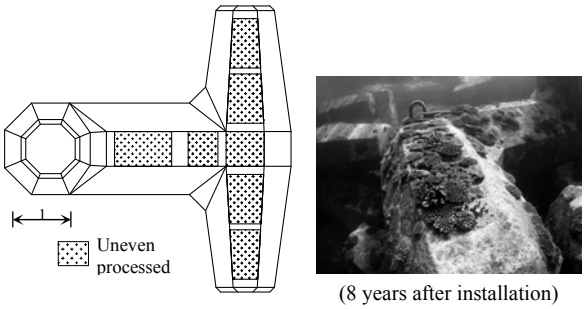
Monitoring of coral settlement and growth on Eco Blocks: experiment to enhance coral recruitment

Eco Block development

To gain an insight into the relationships between substrate properties and coral settlement, we conducted the experiment in two locations using armor blocks with different surface modifications. In 1990, we deployed our blocks with surface protrusions to the height of 1cm onto the side of Naha First Breakwater (primary experiment section), and in the following year of 1991, deployed another type of blocks with triangular or rectangular patterns etched out into the surface onto the side of Naha Breakwater (secondary experiment section). Coral settlement was monitored annually on these blocks by underwater visual observation. The results showed an enhanced attachment of the corals, proving the effectiveness of such modification (Iwakami et al. 1995; Yoshimi et al. 1998). These results in turn saw the development of the "Eco Block," a wave-dissipating block designed to enhance coral settlement, and the launching of an ongoing project using such blocks in Naha Port, from 1999 onwards. The Eco Block's surface (Fig. 1) is unevenly processed in various shapes and patterns when molded in factories.

Monitoring of coral growth on Eco Blocks

The monitoring commenced in 1999 on Eco Blocks placed around Naha Breakwater and continued until 2007. Fixed stations were set up on the top surface of the blocks. Block surfaces were classified into four levels of surface roughness: abrasions in the region of 2mm, 5mm, and 10mm in depth and width, and unprocessed (for control). On the surface of each roughness processed by using some kinds of wood frame while the surface of the concrete was soft, three quadrates (50cm x 50cm) were placed at each depth of C.D.L.-2, -5, and -8m, and the number of colonies, coral cover, number of species and maximum diameter in a quadrate were documented by underwater visual observation.



(8 years after installation)

Figure 1: Eco-block (40t)

Results

Coral settlement and growth on unprocessed blocks

As the fixed monitoring stations were set up on the blocks installed in 1986, the survey marked the twenty-first year in 2007. Figure 2 shows year-on-year changes of coral cover and the number of colonies by water depth. Graphs represent mean values and standard deviation (n=3) of the data obtained from the top surfaces (gradient: 0°).

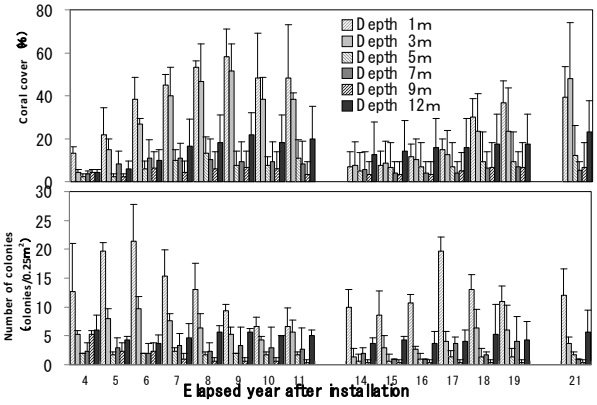


Figure 2: Coral growth on artificial blocks at Naha port. The blocks were installed in 1986. (Mean+SD: n=3)

Changes in number of colonies

At the depths of C.D.L.-1m and -3 m, both the number of colonies and coral cover increased over the period spanning the fourth year (the initial year of the survey) to the sixth year following block installation. Meanwhile, from the seventh to ninth years, the number of colonies was decreasing while the coral cover in the same period was increasing. At the depths of C.D.L.-5m and deeper, the number of colonies remained at relatively low levels, indicating that poorer light conditions in contrast to that at shallower depths (C.D.L.-1 and -3m) slowed coral growth, with minimal competition among the colonies over substrate space.

With regards to the results subsequent to the 1998 bleaching event (the 12th year post installation), no significant changes were observed in colony numbers between the 14th and 16th years. This implies lower

rates of larval recruitment immediately after the bleaching. But in the 17th year, the number of colonies increased, especially at the depth of C.D.L.-1m, with the colonies almost doubling in number. In the 18th year, although the number of colonies dropped notably at C.D.L.-1m, the coral cover showed a continued increase, suggesting the growth of the colonies that had settled in the previous year.

Changes in coral cover

From the 14th year of the survey onwards, the coral cover on all wave-dissipating blocks was lower at all depths than the cover recorded prior to this period This could bear evidence of the loss of live coral cover following the worldwide abnormal increases in sea temperature in 1998. The decrease was of particular significance at the depths of C.D.L.-1m and -3m, where increased sea temperatures must have had greater impacts on the corals. Comparing the results of the 19th and the 21st years, a marked increase in coverage was observed at the depths of C.D.L.-1m and -3m in the 21st year. The results also recorded the highest coverage at the depth of C.D.L.-12m between the 14th and 17th years. This phenomenon is attributed to the difference in the dominant coral species at each depth: Acropora (C.D.L.-1m), *Acropora* and *Pocillopora* (C.D.L.-3m), *Pocillopora* (C.D.L.-7m and -9m), and *Porites* (C.D.L.-12m). The year-on-year changes by depth therefore reflect the recovery processes of these corals. At shallower depths, the *Acropora* and *Pocillopora* species had suffered a reduction in coral cover under the increased temperatures, but showed signs of recovery in later years; while at deeper depths, the *Porites* species survived the period of augmented temperature and continued their growth.

Coral settlement and growth on Eco Blocks

Figure 3 and 4 illustrate the results of coral colony number and coral cover by depth and surface roughness. Graphs represent the mean value (n=3) of the data.

Changes in number of colonies

The number of colonies on the Eco Blocks had been on the rise for all levels of surface roughness at the depth of C.D.L.-2m for two years after block installation. The rougher surfaces demonstrated a greater effectiveness in terms of initial settlement of corals. After the third year, the number of colonies began to decrease on the surface with abrasions of 10mm deep, and it leveled off on the surface with abrasions of 5mm deep. However, on the surface with a roughness of 2mm deep, the number of colonies continued to increase before it plateau in the fifth year. In contrast, colonies on the control blocks showed a slow and gradual increase in number. At the depth of C.D.L.-5, the number of colonies had been largely on the rise for all levels of surface roughness until the fourth year. The greater effectiveness was observed for rougher surfaces as seen in the results from the depth of C.D.L.-2m. After the fourth year, the number of colonies showed a decline trend at all roughness, while the colonies on the control blocks again showed a slow but gradual increase for all surveyed years. At the depth of C.D.L.-8m, the number of colonies were also increasing for over three to four years after block installation but its relationship with surface roughness was not clearly observed.

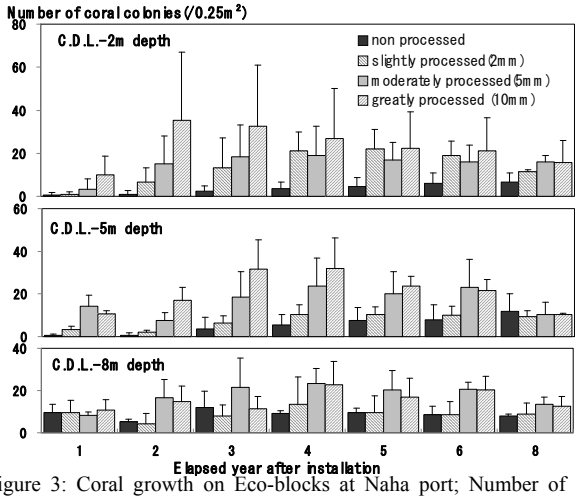


Figure 3: Coral growth on Eco-blocks at Naha port; Number of colonies. The blocks were installed in 1999. (Mean+SD: n=3)

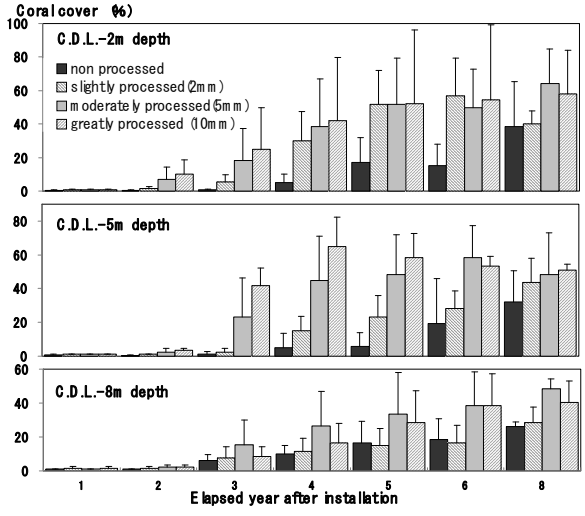


Figure 4: Coral growth on Eco-blocks at Naha port; Coral cover. The blocks were installed in 1999. (Mean+SD: n=3)

Changes in coral cover

At the depth of C.D.L.-2m, the coral cover on the blocks with abrasions of 10mm deep and 5mm deep showed a distinct increase in the third year. In this passage of years, the effects of surface roughness to

coral cover were apparent. After the fourth year, the coral cover on the surface with 2mm-deep abrasions also showed an accelerated increase, reaching closer to those on rougher surfaces towards the eighth year. In parallel, the number of colonies on the processed area showed a decrease, which is probably a result of spatial competition among corals during the colonization process, or the fusion of colonies among the same species. At the depth of C.D.L.-5m, the coral cover on the surfaces with abrasions of 10mm and 5mm hovered at moderate to high levels after the fourth year, ranging between 45% and 65%. On the surfaces with abrasions of 2mm and the control, the coverage for both was gradually increasing after the sixth year, almost rivaling each other by the eighth year. The results from the depth of C.D.L.-8m did not show as major an increase in cover as in shallower waters; however, rougher surfaces (abrasions of 5mm deep and 10mm deep) demonstrated a more rapid increase in coral cover than that on slightly processed surfaces (2mm deep) and the control.

Discussion

Coral settlement and growth on Eco Blocks

The data accumulated on coral settlement and growth processes can be expressed as a curve akin to a logistic curve (Yamamoto, et al, 2002). Taking into account the life cycle of corals, the growth process of corals can be classified into four stages as shown in Fig. 5; 1) recruit period– planula larvae settle on substrates, 2) post-settlement stage– attached planulae develop into polyps, 3) exponential growth stage – budding of polyps affords exponential increase in coral cover, and 4) stable-growth stage. We discuss the effects of uneven surface processing of Eco Blocks on coral settlement and growth at each stage.

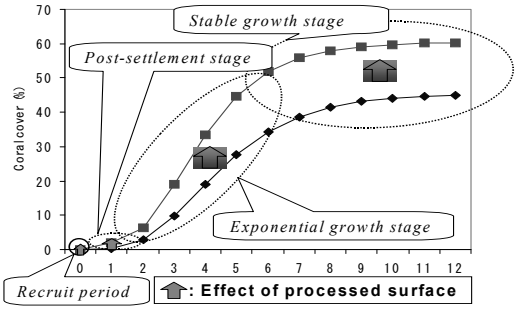


Figure 5: Schematic view of coral growth processes

Larval settlement stage (Recruit period)

Corals release eggs and sperm once in early summer. Fertilized eggs then become planula larvae and drift in surface waters for several days to weeks before attaching themselves to a stable substrate. It is known that the planulae seek out a surface suitable for them to attach to and grow into polyps, and that a top surface of a substrate is seldom sought (Babcock and Mundy 1996). The planula is about 1mm long. Given its tiny dimensions, the uneven processing on the block surface that creates angular sides, if of an appropriate depth, can benefit their settlement to a greater extent than flat surfaces. It is also reported that the roughness on the substrate surface can enhance the attachment of large sea weeds (Terawaki 1988; Watanuki et al. 1990). The reasoning behind this is considered to be the presence of the disturbed flow area and stagnant flow area that are created on the surface with slight abrasions. As such, there is a possibility that these two flow areas created on an unevenly processed surface can physically facilitate the attachment of planulae. This theory could explain the results we obtained in Naha Port, in which larval recruitment and number of coral colonies in the initial growth on the processed area exceeded those on the control.

Initial growth stage (Post-settlement stage)

There is no clear definition that differentiates the initial growth stage from the stable growth stage. However, it has been reported that the growth of newly settled polyps during the initial growth was slow, with higher mortality rates (Wilson and Harrison 2005). The reason behind this is due in part to the presence of predators or competitors in a coral reef ecosystem. Crown-of-thorns and *Drupella* are among the most famous predators, but young polyps are also affected by random grazing by sea urchins such as *Echinometra* and *Diadema* and fish such as parrotfish *Sparisoma viride* (Sanchez et al. 2004) and snail such as *Coralliophila abbreviata* (Williams and Miller 2006).

As these animals also feed on algae that compete with corals for space, some reports have cited that an increase in sea urchins like *Diadema* can result in an increase in coral colonies and coral cover (Macintyre et al, 2005); however, it should be considered that densities of corals, algae and predators dictate their relationships.

With such low chances of survival at this stage, the presence of an unevenly processed surface capable of offering a shelter to newly attached corals against predators, could physically aid in the improvement of survival rates. Also newly-installed structures, which are usually void of large sessile animals or plants that inhibit planula settlement and initial coral growth, could be one of the factors to improve coral settlement and growth.

Exponential growth stage and stable growth stage

Corals grow exponentially at this stage. Therefore, the increase in coral cover seems to depend on coral recruitment in the larval settlement stage and survival

rates during the initial growth stage. The results from the monitoring surveys on Eco Blocks in Naha Port also illustrate the growth with the initial increase in colony numbers and subsequent increase in coral cover. With the increase in coral cover, the number of colonies decreases. It has been reported that mature colonies can be detached or fragmented by stormy waves (Madin and Connolly 2006). Against such external forces, an uneven surface processing that affords a larger surface area and thereby a greater friction force, is expected to allow secure attachment for corals, and thus minimizes their detachment from the substrate.

Eco Block as a restoration technology

After the implementation of wave-dissipating blocks in 1986, the coverage of coral communities exceeded 50% in the eighth year and leveled off until the high temperature-induced bleaching brought about extensive damage to coral communities in 1998. The recovery process shown from 1998 until 2007 illustrates a slower growth of the corals at all depths except for C.D.L.-12m, compared to that before the bleaching.

It has also been reported that coral recovery on old wave-dissipating block, on which dead coral skeletons from the bleaching still remained, was slower than that on the new blocks (Ooka et al. 2006). The space within the coral skeleton serves as habitat for new larval recruits, but it also can be utilized as habitat by coral-predators including gastropods and sea urchins. In addition, some researchers have reported a reduction in larval recruitment after the bleaching events (Jinendradasa et al. 2000; Omori et al. 2001).

In other words, the decreased larval recruitment in the post-bleaching period, and the inhibition of initial larval settlement by mobile benthic predators are considered to be the major factors for the slow coral recovery on substrates after the bleaching.

Meanwhile, when the unevenly processed blocks were compared with the non-processed blocks in Naha Port, the processed blocks showed a significantly greater number of coral colonies. This could imply the possible effectiveness of the surface processing in enhancing the settlement of larvae in conditions where larval supply is poor.

Bachtiar (2000) reported a possibility of coral reef restoration using an artificial substrate. Fox (2005) reported her work in Indonesia's Komodo National Park where she placed artificial substrates among coral reefs having been destroyed by blast fishing and confirmed the steady settlement of *Acropora* species four to five years after the placement.

With all these findings and previous reports, we believe that the placement of artificial substrates with unevenly modified surfaces into the waters of coral reefs suffering degradation can facilitate coral recovery and thus aid in the restoration of coral communities.

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Survival and growth of *Acropora spp.* in mid-water nursery and after transplantation at Phi Phi Islands, Andaman Sea, Thailand

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Abstract. This study aims to determine the survival and growth rates of coral fragments in a mid-water nursery and after transplantation at Phi Phi Islands, the Andaman Sea. A total of 1,120 fragments of two dominant species, *Acropora grandis* and *A. muricata,* were used as seedlings. The average size of fragments was 4.6 cm (SD ± 1.2) and monitoring was conducted monthly. After 4 months in the nursery, survival of fragments was 95.8% and 94.8% for *A. grandis* and *A. muricata*, respectively. There was a significant difference ($p<0.05$) of growth rate in the nursery between *A. grandis* – 0.41 cm mo^{-1} (SD ± 0.21) and *A. muricata* – 0.23 cm mo^{-1} (SD ± 0.20). After 6 months in the nursery, coral fragments were transplanted to a nearby reef which had been destroyed by the Indian Ocean Tsunami in 2004. Survivorship of transplanted fragments after one year was 87.0% for *A. grandis* and 75.3% for *A. muricata*. Growth rates of transplants of *A. grandis* (0.28 cm mo^{-1}) and *A. muricata* (0.38 cm mo^{-1}) did not differ significantly. The relatively high survival suggests that these fast growing corals may be suitable for transplantation in the Andaman Sea area.

Key words: rehabilitation, coral nursery, coral transplantation.

Introduction

The mid-water coral nursery technique was developed in the Red Sea (Shafir et al. 2006) and has advantages in reducing impact to donor colonies, by removing small amounts of coral fragments only, and minimizing sediment smothering to corals in nursery (Shaish et al. 2008). We tested this technique, which has been shown to be successful in a turbid water environment (Putchim et al 2007) to rehabilitate a reef at Phi Phi Islands (Andaman Sea) that was damaged by the Indian Ocean Tsunami in 2004. Survival and growth of two *Acropora* spp. in both a mid-water nursery and after transplantation were studied.

Material and Methods

Study area

The study site was located in the northeast region of the Phi Phi Islands, Andaman Sea, Thailand (7°41.67′ N and 98°41.4597′ E, Fig. 1). A narrow reef with adjacent sandy substrate at approximately 5-18 m depth was chosen. Branching corals of genus *Acropora* especially *A. muricata* and A. *grandis* were dominant species. This reef is a popular diving site and was damaged by the Indian Ocean Tsunami in 2004. The environmental parameters of the area are as follow: annual salinity ranges from 30 to 32 ppt; transparency varies from 7-17 m Secchi depth; and sedimentation rate ranged from 31.2-58.3 $g.m^{-2}.d^{-1}$.

Nursery design

The mid-water nursery was set up at a depth of 12 m within 500 m of the transplantation site at the northeast of the island in May 2006 and corals were reared in the nursery for 4 months until September 2006 (Figure 2). The mid-water nursery was made of 16 modular structures, each consisting of 70 x 90 cm plastic mesh trays attached by cable-ties to rectangular 1.4 x 3.6 m frames made of 1.8 cm PVC pipe. The nursery was comprised of 2 PVC frames, at 1.5 m distance, creating a platform of 4.3 x 3.6 m. The platform was suspended at 6 m depth from four 46 cm mooring buoys which were connected to each other by ropes with small foam buoys sewn at 0.5-m interval. The mooring buoys were held in place by eight 20 kg cement sinkers, each secured to the substrate with 2 anchors.

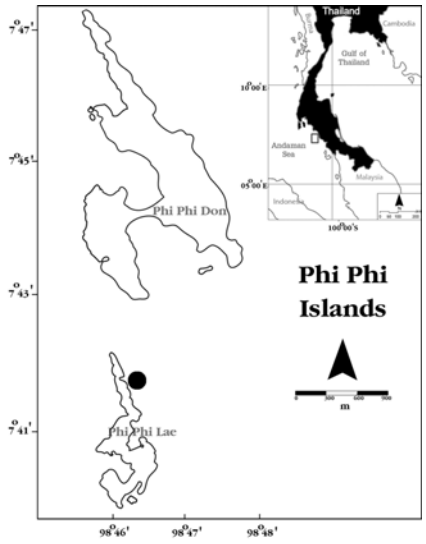

Figure 1: Study area in Thailand, showing the study site

Collecting fragments and transplanting in the nursery
Fragments of two dominant species at Phi Phi Islands, *A. grandis* and *A. muricata*, were collected from the vicinity of the nursery. The average size of the 1,120 fragments was 4.6 cm (SD ± 1.2). Each fragment was inserted into a plastic tube about 4 cm in length, which was attached to the tray mesh (70 pieces per tray). A total of 16 coral trays were fixed onto the PVC platform (Fig 3). Survival and growth were monitored every month for 4 months by recording the status of each fragment as dead, partially dead, bleaching, live, or missing. 24 fragments of *A. grandis* and 57 fragments of *A. muricata* were tagged and measured, by taking under-water photographs of fragment heights with a digital camera using a ruler for calibration.

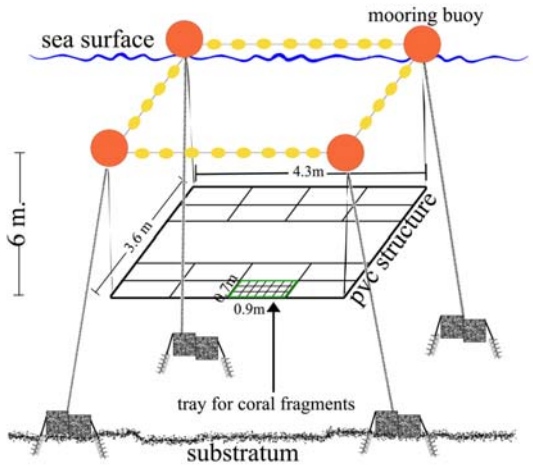

Figure 2: Diagram of floating nursery at the Phi Phi Islands

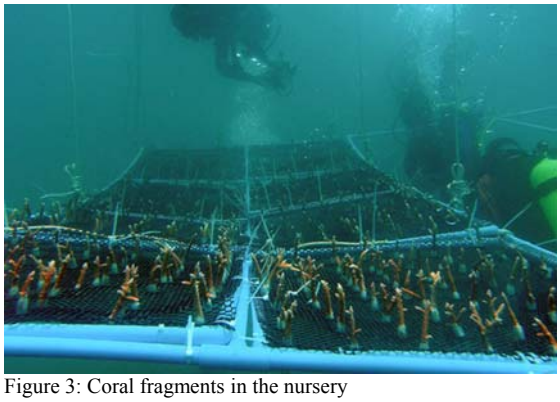

Figure 3: Coral fragments in the nursery

Transplanting to the degraded site
After 6 months in the nursery, the coral fragments were transplanted to a degraded site at 8-10 m depth in November 2006. The fragments were attached to substratum by fastening to metal bars with plastic cable-ties. Those metal bars were welded to a 1-m long metal frame with two legs at either end. Each frame comprised six 15cm long, 1.3 cm diameter metal bars and was secured directly to the substratum which was covered by dead coral rubble. A total of 116 fragments of *A. grandis* (23) and *A. muricata* (93) were transplanted and monitored for survivorship monthly. Growth was measured at beginning and the end (October 2007) of the study period. A subset of eight fragments of *A. grandis* and 18 fragments of *A. muricata* were tagged and measured.

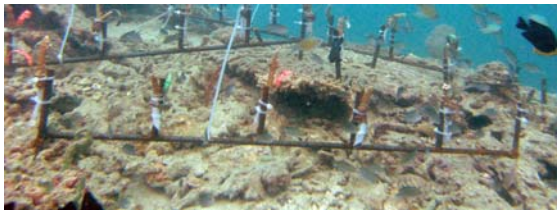

Figure 4: Transplantation method using metal bar attached to the substratum.

Data analysis
Measurement of fragments height was carried out by analyzing pictures taken from the field with Photoshop and Image-Tool software (Shaish et al. 2008). A Student's *t*-test was used to compare growth rates of the two species both in the nursery and after transplantation.

Results
Coral fragments of both species were kept in the mid-water nursery for 6 months, but growth and survivorship were monitored only for the first 4 months. Figure 5 shows the condition of *A. muricata* at the beginning and after one year of transplantation. Survivorship of *A. grandis* and *A. muricata* in the

Table 1: Survival and growth rates of *Acropora grandis* and *A. muricata* in the nursery and after transplantation. Mean and SD are shown for growth rates.

	Coral species	Survivorship			Growth		
		No.	months	%	No.	size (cm)	Growth rate (cm mo^{-1})
Nursery	*A. grandis*	210	0			4.4±0.7	
		192	4	95.8	24	6.1±1.2	0.41±0.21
	A. muricata	842	0			4.6±1.4	
		797	4	94.8	57	5.5±1.6	0.23±0.20
After transplantation	*A. grandis*	23	0			7.1±1.6	
		20	12	87.0	8	10.2±3.7	0.28±0.24
	A. muricata	93	0			5.7±1.9	
		70	12	75.3	18	9.9±3.6	0.38±0.31

nursery was 95.8% and 94.8% respectively after 4 months and growth rates were 0.41 cm.mo^{-1} and 0.23 cm.mo^{-1} respectively (Table 1). The growth rate of *A. grandis* was significantly higher than that of *A. muricata* (*t*-test, $p<0.05$).

Survivorship and growth rates of coral fragments of both species transplanted to the degraded reef area were monitored for 12 months. Survivorship of transplanted fragments was 87.0% for *A. grandis* and 75.3% for *A. muricata*. Growth rates were 0.28 cm.mo^{-1} for *A. grandis* and 0.38 cm.mo^{-1} for *A. muricata* (Table 1) and were not statistically different (*t*-test, $p>0.05$).

One year after transplanting, most of transplanted colonies still did not attach to the metal bars with only one out of 26 monitored colonies becoming partially self-attached (Fig. 6).

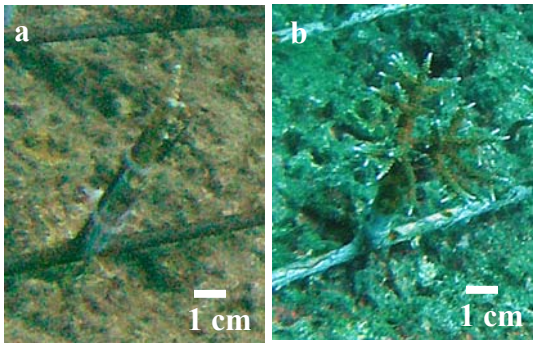

Figure 5: (a) An *Acropora muricata* transplant at the beginning of transplantation; (b) the same transplant one year after transplantation.

In August 2008, two years after transplantation, the site was also visited. About 50% of the transplanted colonies had grown to fill the 20-cm spaces in between the metal bars. However, some colonies were still at the initial transplanted size and a few colonies were missing or dead.

Figure 6: A transplanted colony partially self-attached to a metal bar after one year.

Discussion and Conclusion

Rearing of corals in mid-water nurseries is a relatively new technique (Shafir et al. 2006). The results from this study in the Andaman Sea and those of Shaish et al. (2008) from the Philippines indicate that the technique can be beneficial in areas other than the Red Sea (Shafir et al. 2006). The present study shows promise in an area with relatively high sedimentation (Putchim et al. 2007). In the Andaman Sea, we had to use larger initial fragments than those in the Red Sea because of higher turbidity and sedimentation. For example, small-sized (1 cm) *Pocillopora damicornis* fragments survived well in a mid-water nursery in the Red Sea (Shafir et al. 2006) but did not survive in turbid coastal waters of Cape Panwa, Phuket Island, Andaman Sea (Putchim 2007). Sedimentation rates at Cape Panwa varied from 200-500 g.m^{-2}.d^{-1} whereas those in the Red Sea varied from 1.8-8.4 g.m^{-2}.d^{-1} (Bongiorni 2001). In this study at Phi Phi Islands which has moderate sedimentation rates (ranging from 31.2 ± 2.7 g.m^{-2}.d^{-1} to 58.3 ± 1.6 g.m^{-2}.d^{-1}), the initial mean height for both species was 4.6 cm. This size appeared adequate to ensure survival.

This study showed high rates of survivorship in *A. grandis* and *A. muricata* in the nursery and after transplantation. The mean growth rate of *A. muricata* after transplantation (0.38 cm.mo^{-1}) was similar to that of *A. muricata* in transplantation at Cape Panwa, Phuket (0.3 ± 0.3 cm.mo^{-1}) (Yucharoen et al. 2008)

and growth rate in Okinawa (3.5 $cm.yr^{-1}$) (Okubo et al. 2005). Whereas the growth rates of *A. muricata* in the natural environment of the Andaman Sea showed seasonal variation ranging from 0.3 $cm.mo^{-1}$ during the SW monsoon to 0.9 $cm.mo^{-1}$ during the NE monsoon seasons (Charuchinda and Hylleberg 1984; Chansang et al. 1992). Growth of *A. muricata* in our study was less than that obtained using the same technique in the Philippines (0.8 $cm.mo^{-1}$) (Shaish et al. 2008). The practice of using metal bars for fixing transplanted fragments to substratum should be reviewed as growth of live tissue over the metal was very limited and adding foreign objects into the reef environment should not be encouraged. However, the metal frames helped to secure transplanted colonies until they were large enough to withstand the surrounding water movement. After 2 years, the frames were not very visible as transplanted colonies had grown to cover the frames.

In conclusion, fragments of *A. muricata* and *A. grandis* had high survivorship both in nursery and after transplantation, thus it is recommended that these coral species are suitable for nursery and transplantation in the Andaman Sea.

Acknowledgement

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Coral transplants as rubble stabilizers: a technique to rehabilitate damaged reefs

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Abstract. Developing practical techniques to mitigate the effects of coastal development, ship groundings and destructive fishing has challenged reef managers for 30 yr. Coral communities reduced to rubble do not support recruits due to their instability; such areas have slim chances of recovering naturally. We relocated "corals of opportunity" to a 50-year-old rubble bed and tested the efficacy of using two species of corals with contrasting morphologies as rubble consolidators. We hypothesized that *Porites rus,* which forms upright columns and extensive basal plates, would be a superior consolidator to *Porites cylindrica,* which has a dendritic growth form without basal plates. After 18 months, growth and survival varied significantly between species. The majority of *P. rus* transplants survived (93.3%), while only 23.3% *P. cylindrica* transplants did. Mean basal growth was 0.8 mm mo^{-1} for *P. rus* and 0.07 mm mo^{-1} for *P. cylindrica,* with *P. rus* overgrowing rubble within 6 mo. The plate-forming morphology enhanced survival by stabilizing fragments as they grew. In contrast, none of the *P. cylindrica* transplants consolidated onto rubble during the course of our study.

Key words: rehabilitation, *Porites,* rubble stabilization, Guam.

Introduction

Guam reefs have traditionally been heavily used for food and recreation (Amesbury and Myers 1982; Zeller et al. 2006) and fish stocks have declined steadily over the past 50 yr. The Guam Visitors Bureau reported a record high of 1 million Asian tourists in 2004, a dramatic increase over past years despite economic setbacks (GVB 2004). Physical damage to reef structure from water sports is associated with premier tourist destinations (Hawkins and Roberts 1993; Rouphael and Inglis 2001; Burdick et al. 2008), increasing coral mortality and loss of reef structure (Edwards and Clark 1998; Bowden-Kerby 2003). Compounded by chronic nutrient inputs, sedimentation and pollution, and acute outbreaks of the Crown-of-Thorns starfish (Birkeland and Lucas 1990), recovery of Guam reefs has been slow to non-existent. A further complication is low coral recruitment (0.14 recruits/15-cm^2; Birkeland et al. 1981, 0.002 recruits/15-cm^2; Birkeland 1997, 0.013 recruits/15-cm^2; Minton and Lundgren 2006). The reasons for this are unclear and therefore difficult to mitigate. If Guam reefs are self-seeding due to minimal larval flow between islands, declines in coral cover will be self-perpetuating (Porter et al. 2005).

Restoration ecology explores approaches to mitigate damage to ecosystems, promote recovery, and reestablish specific attributes and function (Rinkevich 2005). Techniques currently employed range from coral gardening and transplantation to deploying artificial structures for recruitment substrate and habitat. In Guam, coral transplantation has been conducted since the late 1970s with various objectives, which have included: reestablishing coral communities in thermal effluent areas (Birkeland et al. 1979), preserving rare coral species (Plucer-Rosario and Randall 1987), translocating corals due to underwater construction and ship groundings (Naughton and Jokiel 2001), and mitigating coral loss from fiber optic landing development (Kolinski 2002). However, the challenging issue of persistent rubble fields from physically destructive events has been largely ignored. As Guam reefs will be subjected to increasing coastal and military development, continuous boat activity, and periodic typhoon surge, this problem requires urgent attention.

Despite the innate ability of corals to fragment, survival depends on attachment to stable substrate (Bothwell 1982). Corals can recover from natural calamities such as storms, but chronic fragmentation by human activity creates rubble fields which fail to consolidate because recruits do not survive. Practical approaches to rehabilitate rubble and facilitate coral reestablishment are few. Fox et al. (2005) and

Raymundo et al. (2007) found high transplant survival and recruitment on rubble stabilized by combinations of plastic mesh, rock piles and cement slabs.

This investigation tested the use of coral fragments to consolidate rubble while simultaneously relocating corals from a marginal habitat scheduled for dredging. These 'corals of opportunity' (Edwards and Gomez 2007) were a mixed community of *Porites rus* and *Porites cylindrica*. We compared the rubble stabilizing ability of plate-forming *P. rus* with the upright branching *P. cylindrica.* We predicted that *P. rus* would be a superior species to consolidate underlying rubble than *P. cylindrica* due to its morphology and rapid growth rate (Veron 2000).

Materials and Methods

Study site

The experiment was conducted on Sumay Mound, a nearshore seamount rising from 30 m to 15 m depth, 1 km south of the Apra Harbor inner channel dredge site. Approximately 20% of this reef was dominated by rubble from anchor damage in the 1950s. Recovery, to date, has been insignificant. Further, the rubble bed is seasonally dominated by the foliose macroalga *Padina sanctae-crucis* such that live hard coral cover constituted only 15% of the substrate (Fig. 1). The persistent rubble, protection from typhoon surge and proximity to the source site were attributes favorable for our recipient site.

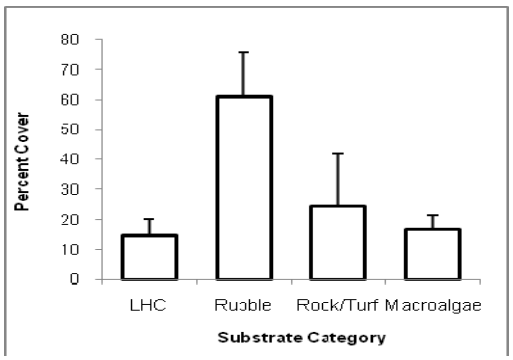

Figure 1: Benthic composition within the rehabilitation site on Sumay mound, showing a dominance of coral rubble.

Coral translocation and establishment

Fifteen colonies each of *P. rus* and *P. cylindrica* were transported from the channel to Sumay Mound and allowed to acclimate for 12 weeks. Three 14 m^2 areas within the rubble field were manually cleared of macroalgae. Donor colonies were then fragmented into sizes of three to four branches and cemented upright to three rubble pieces using Z-Spar Marine Epoxy®. This fragment size provided refuge from size-specific mortality (Hughes and Jackson 1985; Harriot and Fisk 1988) and the rubble provided weight and stability. Twenty such fragments (10 per species) were positioned in rows within each plot, with the two species alternating within each row. Transplant performance was monitored at regular intervals for 18 mo. Weeding of *Padina sanctae-crucis* was undertaken as necessary and fragments that had become overturned or detached were repositioned and re-epoxied. Yap et al. (1992) found that corals transplanted during warm months survived and grew less than those transplanted in cooler months. Therefore, we monitored temperature using Stow-away Tidbits (Onset Corp.®).

Transplant performance was characterized per species as timing of basal attachment to substrate, basal growth (mm mo^{-1}), disease signs, tissue loss or compromised health (bleaching, algal overgrowth), recovery from partial mortality, and full mortality. Basal growth was expressed as mean maximum basal width in millimeters. Disease prevalence was calculated as the number of fragments showing disease / total fragment number * 100. As differences between plots were insignificant, data for each species were pooled for analysis using unpaired *t*-tests.

Results

Porites rus consistently demonstrated better overall performance than *P. cylindrica,* by having low partial mortality, high survival, less disease and high consolidation rates via rapid basal growth. Per species mean responses are summarized in Table 1.

Table 1: Summary of transplant performance, mean ± SD.

Performance parameter at 18 mo	Species	
	P. rus	*P. cylindrica*
% survival	93.3±0.05	23.3±25.1
% with partial mortality over 18 mo period	67.0±20.8	96.6±5.7
% with tissue regrowth at 18 mo	56.6±15.7	0
Mean basal width (mm)	62.8±6.1	37.4±6.7
Total basal growth (T_{final}-$T_{initial}$)	13.5±0.6	-1.2±0.20

Basal consolidation and growth

It took approximately three months for coral tissue to begin to overgrow the epoxy and rubble pieces. At this time, 74% of *P. rus* transplants showed initial cementation onto substrate, while only 27% of *P. cylindrica* transplants showed this response. By the end of our monitoring period, 10% of *P. cylindrica* fragments had attached to underlying rubble, as opposed to 62% of *P. rus* fragments. Basal growth rate averaged 0.75 mm mo^{-1} for *P. rus*, and 0.07 mm mo^{-1} for *P. cylindrica*, for the months when there was positive growth (t=8.51; p<0.001).

Partial mortality

Partial mortality involved tissue loss which usually began at the fragment base and progressed upward. Recovery involved tissue re-sheeting over dead skeleton. When this did not occur, skeleton was rapidly colonized by algae, resulting in additional tissue loss over time. While it was difficult to determine the causes of mortality, bleaching and coral diseases (ulcerative white spots and white syndrome; Raymundo et al. 2003; Willis et al. 2004) were observed periodically. Seasonal accumulation of *Padina* around the base of fragments resulted in bleaching of underlying tissue; this was the most common cause of tissue bleaching and loss. An average of 23% of *P. rus* transplants incurred partial mortality within the first three months and by the end of the study period, 67% had exhibited some tissue loss but 57% of these showed some tissue resheeting over the dead skeleton (Table 1). Partial mortality for *P. cylindrica* on the other hand, increased rapidly from 27% at 3 mo, to 97% by 18 mo (*t*-test of mean mortality between species; p=0.0164). Thus, whereas 57% of *P. rus* transplants that lost tissue showed recovery, *P. cylindrica* fragments with partial mortality died.

Full mortality

Overall, *P. rus* survived better to 18 mo than did *P. cylindrica* (Table 1, Fig. 2; *t*-test p=0.041). Sources of mortality were the same as those mentioned above, though while *P. rus* appeared to recover more frequently, *P. cylindrica* transplants more often showed a general decline in health over time with accompanying tissue loss and algal overgrowth. Further, because they failed to consolidate around the base of the fragment, overturning, detachment and subsequent abrasion and tissue loss were also causes of mortality.

Coral disease

Ulcerative white spots disease affected *P. cylindrica* much more than *P. rus* (*t*-test p=0.014), and most severely within the first three months after translocation. UWS prevalence reached a high of 23% at 3 mo in *P. cylindrica* transplants, though infected fragments showed subsequent recovery and none died of the disease. UWS was observed on *P. rus* at two census visits, October 2006 and March 2007, and all fragments recovered by the next census. What appeared to be putative white syndrome lesions appeared on several *P. cylindrica* fragments, though these lesions usually became fouled with algae by subsequent census visits and did not appear to progress. There was no evidence of a link between prevalence of either disease and seasonal warming seawater temperature.

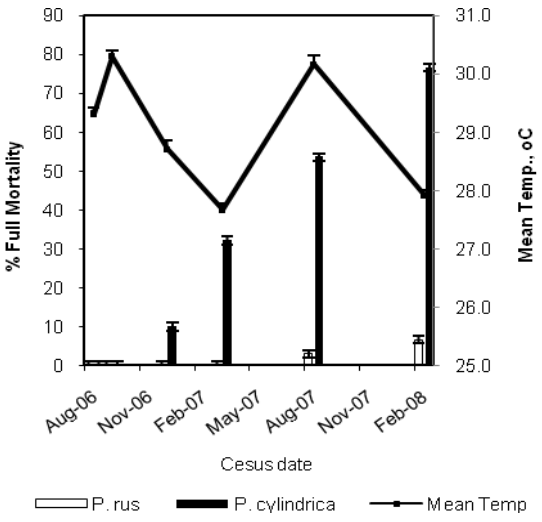

Figure 2: Cumulative mortality over time in two species of transplants, plotted against mean weekly water temperature. N=20 fragments per plot; mean ±SD

Temperature seasonality effects

Water temperature showed a seasonal trend; highest temperatures were recorded during August (31.2°C) and coolest temperatures occurred in January-February (27.5°C; Fig. 2). However, although bleaching was recorded at other sites around Guam during the warm season, no bleaching was observed in our transplanted population. Further, our data suggested no correlation between warm water and any of the performance parameters we measured.

Discussion

Our results showed that live coral transplants can stabilize rubble in low-energy environments. The morphology of our two closely-related species affected their ability to consolidate rubble, which was key to their survival and establishment. *P. rus* was superior in overall performance, demonstrating low partial and full mortality, more frequent recovery, less susceptibility to disease, and greater basal growth and consolidation compared to *P. cylindrica* (Fig. 3). Yap et al. (1998) noticed a similar response; *P. cylindrica* fragments showed significantly more partial or full mortality at one year post-transplant than other test species. However, not all of our success measures can be attributed to morphology alone, suggesting that *P. rus* may have greater acclimatization capability.

The growth rates we observed were lower than rates for other species reported in the literature. Raymundo (2001) reported an increase in fragment surface area of 5 cm^2 wk^{-1} in *Porites attenuata,* which is closely related to *P. cylindrica.* Similar rates were observed in *Acropora hyacinthus* transplants by Yap et al. (1992; 6.9-117 cm^2 mo^{-1}) and *Pocillopora damicornis* (~2.65 mm mo^{-1} linear growth; Birkeland 1979). However, we report here only basal extension

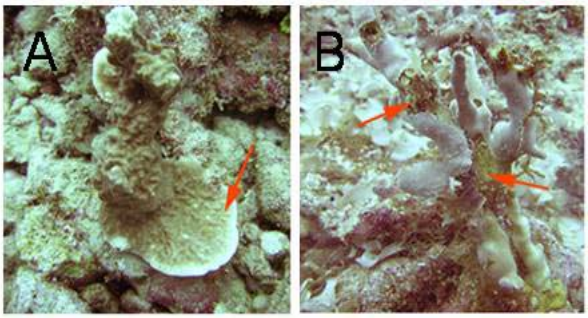

Figure 3: Transplants of (A) *P. rus* and (B) *P. cylindrica* at 18 mo. *P. rus* shows basal plate formation (arrow) and consolidation on rubble; *P. cylindrica* shows tissue loss and algal overgrowth (arrows).

as the relevant growth parameter for our study, as consolidation rate was the metric of concern. As basal plates are generally circular to ovoid in area, this translates into substantial surface area. For instance, mean maximum basal width for *P. rus* transplants at 18 mo was 7.3 cm. Using the formula for surface area of a circle, this would provide roughly 44 cm^2 of basal skeleton cemented onto the underlying rubble. An increase in maximum width by 1 cm would translate into an increase in surface area of 12 cm^2. Although these figures are rough estimates, they provide some idea of the rate at which *P. rus* would cover rubble substrate over time.

Mortality was higher in *P. cylindrica* and most prevalent during seasonal abundances in *Padina* spp. The alga overgrew the base of most fragments. Direct contact of corals with macroalgae is energetically costly (McCook et al. 2001), and diverts resources for growth toward tissue repair (Lirman 2001). Although both species were equally susceptible to algal overgrowth, *P. rus* more often recovered after initial contact with algae.

In Guam, *P. rus* dominates many coral communities, often occupying ~ 80% of the substrate (L. Raymundo, pers. obs.). This species demonstrates several traits which contribute to its dominance: an ability to rapidly co-opt space, outcompete other species (such as *P. cylindrica;* Caballes 2006), low susceptibility to disease (Raymundo et al. 2005). Holbrook et al. (2002) also noted that *P. rus* provides superior fish habitat to massive *Porites* species. All of these traits point to its suitability in rehabilitation efforts. However, it is important to bear in mind that the establishment of single-species stands should not be a goal of rehabilitation. Hardy, plate-forming species may be suitable for initial stabilization efforts, but other species should be introduced later to increase species and structural diversity.

Stabilized coral rubble provides a suitable substratum for recruitment and for asexually-produced fragments (Bothwell 1982; Fox et al. 2005). Raymundo et al. (2007) noted higher recruit survival on stabilized, rather than loose, rubble and recruits then further consolidated rubble as they grew. Stabilizing rubble also eliminates further damage to the reef, as when unconsolidated rubble shifts during storms (Clark and Edwards 1995; Fox et al. 2003). Finally, as rubble is replaced by live hard coral, macroalgal abundance may decline. This effect is particularly visible during periods of seasonal algal abundance (Gleason 1999).

Ulcerative White Spots (UWS) was first described as affecting the genus *Porites*, but has recently been observed on several other genera (Raymundo et al. 2005; Kaczmarsky 2006). The pattern of occurrence we observed suggested a link with fragment stress rather than seasonal temperature trends; transplanted fragments may have been stressed, and their immuno-defense capacity compromised, by the transplantation process. A similar pattern was observed in fragments of *P. attenuata* transplanted to a site with poor water quality in the Philippines, when the disease was first observed and described (Raymundo 2003). Disease is a potentially devastating source of mortality for transplants, and transplantation a potential source of disease introduction. To date, these issues remain untested but suggest a line of research that could improve transplant survival and be utilized to improve best practices for rehabilitation efforts.

Persistent rubble fields remain a challenge for restoration and are often ignored in favor of efforts which may result in higher survival. However, destructive fishing and boat groundings continue to create patches of "dead space" in reef communities. In the Philippines, patches created by destructive fishing practices banned 20 or 30 years prior persist with negligible coral growth (Raymundo pers. obs. and fisher interviews). Thirty years ago, Alcala and Gomez (1979) predicted dynamite-blasted reefs would take 50 yr to recover 50% of their original coral cover; Riegl and Luke (1998) increased the predicted regeneration time to several hundred years. Clearly, persistent rubble is an issue increasingly difficult to ignore. However, this source of damage frequently occurs at a scale at which many rehabilitation efforts currently operate (10^{-2} to 10^{-1} ha; Edwards and Gomez 2007). Therefore, efforts to develop methods to encourage regeneration will likely result in success.

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Large coral transplantation in Bal Haf (Yemen): an opportunity to save corals during the construction of a Liquefied Natural Gas plant using innovative techniques

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Abstract. As part of a mitigation measure associated with the construction of a Liquefied Natural Gas plant, four large coral transplantations were carried out in Yemen between January and October 2007. Around 1,500 selected coral colonies were removed from areas to be impacted, transported and cemented in new sites. Transplanted colonies belong to 36 species and 25 genera. Among these, 140 large *Porites* spp. weighing from 200 kg up to 4 tonnes, were moved using new transplantation techniques. Growth, *in situ* mortality and health of the transplants were monitored over one year using photo quadrats, close-up pictures and linear growth measurements. Overall, survival of corals one year after transplantation was 91%. Most losses of transplants were apparently due to sedimentation of fine particles in the transplanted areas, fish predation, fisher activity and swell effects. Evidence of coral growth after transplantation was observed, especially in *Acropora* and *Porites* species, and on some faviids. The transplantation results demonstrate the capacity of corals to adapt to a new environment, in favorable conditions. They show that carefully designed coral reef rehabilitation strategies can be part of industrial development processes, whenever necessary.

Key words: coral transplantation, reef restoration, mitigation measures.

Introduction

The Yemen Liquefied Natural Gas (YLNG) project is a $5 billion project to build a pipeline and a liquefaction plant to process and ship natural gas from Yemen to the world markets. In the plant the gas is compressed and liquefied at minus 160°C and transported via sea tankers. YLNG's environmental approach is: first to eliminate or mitigate impacts by redesign when this is possible. If elimination or mitigation is not effective or possible, YLNG policy is to compensate for impacts, and to provide investment to promote sustainable improvement in marine environmental conditions, and monitoring of the marine environment to ensure that these measures are effective. Creocean carried out an Environmental Baseline Study (EBS) in 1997 and 2005. Up to that time, knowledge of corals in the region was limited to a few published references (Sheppard et al. 1992; Kemp and Benzoni 1999; Kemp and Benzoni 2000; Benzoni et al. 2003). The EBS showed that Bal Haf cape where the LNG plant is being constructed was characterized by a high coverage of diverse corals, abundant and diversified fish, and highly three-dimensional coral communities. As part of the mitigation measures, a coral transplantation was proposed to save coral colonies from destruction on sites where the marine works would take place.

Three localised areas with dense coral communities will be impacted by the construction works (Fig. 1). On the North side: 1) the Intake area where an intake water cooling pipeline will be placed to pump deep water, 2) the Jetty area where a loading jetty will be built to receive tankers, and 3) the Golf area, where a shoreline protection with concrete blocks is required. On the South side, one area will be impacted: the Outfall site where a pipeline is to be placed to discharge warm seawater after it has been used for cooling during the gas liquefaction process.

Different sites were chosen to receive the transplanted corals (Fig.1). Selected sites met the following criteria: they were close to the original sites but out of the impacts of the plant construction (the selected sites were from 100 to 1100 m away from the donor sites), and were characterized by similar depth, hydrodynamic and water quality conditions.

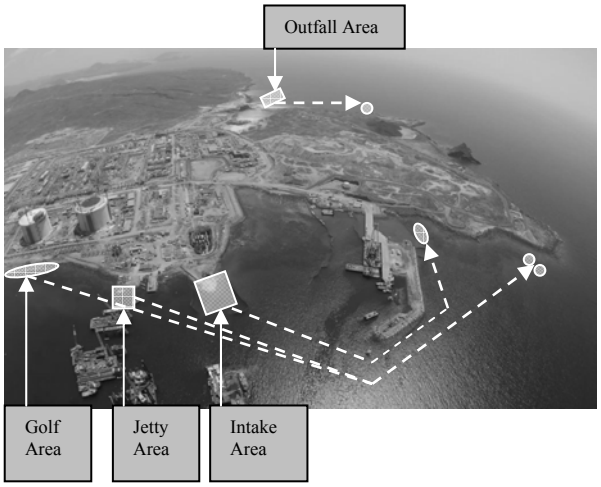

Figure 1: Aerial view showing the donor and the receiving sites.

A study of the coral communities on the donor sites revealed domination by the branching *Stylophora* with presence of large massive *Porites lutea* and *P. lobata*, and different faviids, especially *Platygyra daedalea* and *Favites* spp. A few tabular *Acropora* were also found. Because these areas were too large to be integrally transplanted and to increase the chances of coral survival, it was decided *a priori* to transplant selectively the largest colonies, the rare or uncommon species, the slow growing species, and only the colonies in good health. Edwards and Clark (1998) argued that there has been too much focus on transplanting fast-growing branching corals instead of slowly recruiting massive species, which generally survive transplantation well but often recruit slowly.

Material and Methods

The variety of colony shape and size among the transplanted corals required different methodologies of collection, transport and attachment. The small to medium-sized colonies (40–60 cm diameter) were removed using hammer and chisel and placed in pierced plastic baskets underwater. The baskets were loaded on a boat and directly transported to their final location (Fig. 2). They were protected from the sun and wind by a plastic cover and regularly splashed with fresh seawater. The medium to large-sized colonies (0.6–4 m diameter) were removed using a crowbar. Those colonies ≤1 m diameter were placed in a large steel basket (2 m^2 [base] x 100 cm [high]) directly underwater, which could be raised to the surface with lifting balloons and towed under the surface by boat. The large *Porites* colonies (1–4 m diameter and up to 4 tonnes) were drilled to a 20 cm depth in order to fix with epoxy resin one or two (12 or 18 mm) stainless steel screws into the skeleton, which could be attached to a lifting balloon (Fig.2). Each of these colonies were then lifted to the surface and slowly towed by boat to the transplantation site.

All the corals colonies were cemented at their final location to avoid damage by swell or fish predation. Epoxy was chosen for the fragile colonies such as branching *Acropora* species. It took around half an hour for the cement bond to become strong and a few hours to be totally hard.

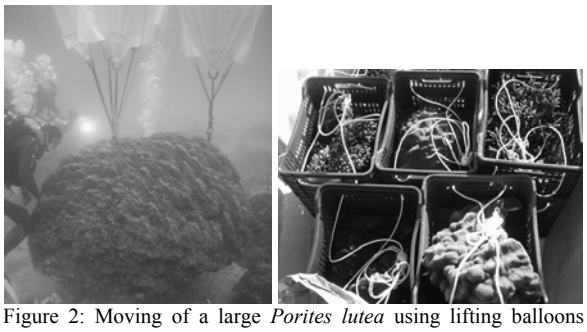
Figure 2: Moving of a large *Porites lutea* using lifting balloons (left), transport of small colonies in baskets out of water (right).

A monitoring program was set up to survey the different transplantation sites and to measure the adaptation of corals to their new environments. Every four to six months (2007: January, May, October; 2008: February, June) a digital image mosaic was taken of each entire site, after installing a grid. Survival and health (occurrences of diseases or damage) were recorded for each colony.

Close-up pictures and measurements using large calipers were used to survey the health of a dozen selected colonies per site, with quadrats being used to follow the growth of 10 tabular *Acropora*, 15 branched *Acropora*, 17 massive *Favites* and 24 *Porites lutea*. Plastic collars fixed to the colonies at the beginning of the monitoring were used as references for diameter, radial, or branch extension measures. Stainless steel cables were also installed on the surface of massive *Porites* to monitor their growth by coral tissue extension on the cable.

Results

1,495 coral colonies belonging to 11 families (Table 1), 25 genera and 36 species were removed from sites to be impacted and transported to new areas safe from the effects of the construction works. The taxonomic composition of the transplants (Table 1) was dominated by faviids with a large number of *Platygyra daedalea* and different species of *Favites*. A lot of Poritidae were translocated as well, with a special care taken in the case of 140 particularly large colonies (1–4m in diameter), weighing from 200 kg up to 4 tonnes .

Table 1: Number of transplanted colonies per scleractinian family and relative percentage

FAMILIES	NUMBER	%
Acroporidae	113	8.0
Agariciidae	71	5.0
Dendrophylliidae	7	0.5
Faviidae	750	50.0
Fungiidae	10	0.5
Mussidae	17	1.0
Oculinidae	12	0.5
Pocilloporidae	102	7.0
Poritidae	376	25.0
Psammocoridae	7	0.5
Siderastreidae	30	2.0
TOTAL	**1,495**	**100.0**

Fourteen months after the first transplantation operation in January 2007, 91% of the transplants were alive and healthy. The survivorship for each operation is shown in Table 2.

Table 2: Survivorship rate (%) of the transplantations depending on the duration of survival.

Area	Total (n)	Duration of survival (month)			
		3 (%)	5 (%)	10 (%)	14 (%)
Intake	608	99	-	96	94
Jetty	400	-	89	82	
Golf	79	-	78	74	
Outfall	408	-	100		

From the first monitoring after transplantation, there was some evidence of growth of the corals. Growth was observed at the base of the colonies where the living tissue overgrew the cement, the epoxy or the substrate directly. Colonies of the genus *Acropora* showed the most rapid growth, especially for the tabular colonies, with an average diameter extension of 1 cm mo^{-1} (± 0.4 cm).

Growth was also visible on the large *Porites lutea* on which stainless steel cables were placed. These were quickly covered by living tissue. A few samples were collected and analysed by tomography to determine the rate of growth of these corals.

Various types of damage occurred to corals after transplantation. An invasive red sponge (*Clathria* sp.) attacked corals at all sites, especially massive *Porites*. Its fast expansion induced considerable localised damage at transplanted sites, killing the coral by its growth over the living coral tissue. This phenomenon has also been recorded in other areas of the Gulf of Aden by Benzoni et al. (2008). Infestation by pyrgomatid barnacles was also recorded especially on *Porites* colonies and seemed to be seasonal and may possibly have been linked to the sedimentation at the sites. It was observed as well on non-transplanted corals. Fishermen caused additional damage. Swell and current induced coral damage occurred in July and August during the monsoon season. The use of cement with an adjuvant to keep it compact underwater was successful for most of the corals. Only three small colonies became detached after one year, but 17 out of the 140 large *Porites* were moved by the strong swell at the most exposed site (Fig.1, close to the cape). Rubble, sand and gravel transported by swell waves covered some of the branching colonies.

Fish were responsible for some coral damage, during the attachment phase. The large wrasse *Coris formosa* turned over the corals to eat boring shellfish living inside the coral skeleton and revealed by the collection process. Damage of *Porites* by the giant hogfish *Bodianus macrognathos* was also observed. Most of the loss transplants, especially on one of the areas where a large part of the transplanted corals belong to the Acroporidae (*Montipora* sp*.)* and the Agariciidae (*Pavona cactus)* were due to fish predation. Parrotfish and triggerfish scraped and broke parts of the coral colonies during feeding or nest-building respectively. This damage occurred to both natural colonies as well as the transplants, but the fish appeared more attracted by stressed corals than by healthy ones.

Finally, sediment deposits, which formed on the coral colonies, induced necrosis on top of massive corals such as *Platygyra daedalea* and *Favites* spp. in the transplant area closest to the plant construction works.

Discussion

The use of a screws drilled directly into the coral skeleton to fix to lifting balloons was an innovative technique to transport large corals. It allowed corals to be protected from damage during the collection and the transport phases (for example, abrasion of the living tissue by lifting belts usually used to move large corals). Moreover, it allowed the corals to remain underwater during translocation and allowed them to be positioned precisely on the receiving reef. No published references were available to compare the success of this technique because it is the first time such large corals have been successfully moved. Only references on the transplantation of small colonies (10–20cm) were found (Clark and Edwards 1995; Alcala et al. 1982; Plucer-Rosario et al. 1987; Yap et al. 1992) and these workers all used plastic baskets for underwater transports. All reported the same problem: considerable loss of transplants from higher energy sites, whatever methods of attachment. Following these results, we decided to cement all the transplants, even the heavy colonies (up to 1 tonne) and we only had loss due to swell at the most exposed site. This points out that the location of a relocation site is one of the most important variables in a transplantation operation.

The differences in survivorship between our different operations (from 74% to 94% over one year) are most likely due to effects of the monsoon. During the summer monsoon, extreme oceanographic conditions occur, with strong swell, current and wind, and an upwelling carrying cold water at around 17°C (Sheppard et al. 1992). We conclude that corals which were removed and transplanted just before the monsoon season did not have enough time to recover and adapt to their new environment before they faced adverse sea conditions. Indeed, the transplantation process, even if it is done well, is likely to be stressful for corals (Clark and Edwards 1995). The transplants need time to recover to be able to resist new stress. This may be why they seemed to be susceptible to predators and diverse attacks such as fish, red sponge or pyrgomatids. Survival in this study appeared relatively high compared to previous studies. For example, Alcala et al. (1982) obtained 40% survival of transplants after one year, Auberson (1982) 70%, but 20 to 50% on high-energy shallow sites, and Clark and Edwards (1995) 75% survival. This was probably partly due to the focus on larger, massive and sub-massive species in this project.

To contend with fish predation, we adapted our methodology, for example attaching the corals directly after their removal. Fish may be more attracted by stressed corals, perhaps due to the increase in the mucus production. The selection of corals in good health before transplantation is very important to limit these attacks.

To conclude, the 91% survival after more than one year is encouraging but other surveys are needed to complete these data. We can determine the best period of the year to move corals, depending on the season and the weather conditions: for Yemen waters it seems to be during the months following the monsoon. According to other authors, the species that better support the transplantation seem to be the massive ones such as the Poritidae and the Faviidae. The best way to move corals depends on the species, the size and the distance they need to be translocated. Large colonies can be towed underwater by boat and small ones transported out of water in pierced plastic baskets.

These operations show that it is possible to do something to save part of the reef when a construction work is necessary. It is also possible to use these transplantation techniques during the building of industrial plants, hotels, harbor extension, or any construction that will damage part of the reef for coastal development. The cost of mitigation appears relatively modest. As an example, the cost of this operation represents less than 1% of the total amount of the plant construction.

Acknowledgement

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Transplantation of *Porites lutea* to rehabilitate degraded coral reef at Maiton Island, Phuket, Thailand

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Abstract. This study compared the growth and survival of different sized transplants of *Porites lutea* at Maiton Island, Thailand. Fragments in three different sizes, 5.0 x 5.0 cm (large), 3.5 x 3.5 cm (medium) and 2.5 x 2.5 cm (small), were detached from intact coral colonies. Unattached coral colonies in each size category from the site were also transplanted for comparison. The fragments and unattached colonies were cemented on concrete blocks. Medium-size fragments and colonies showed high survivorship whereas small-size fragments and unattached colonies showed low survivorship. Growth was measured as increase in colony plan area and increase in height. Area of transplants increased exponentially and the growth constant of small-size colonies was significantly higher than that of large-size colonies (S-N-K, p = 0.021). Rates of height increase were significantly different among all sizes for fragments (with smaller fragments performing more poorly) whereas there was no difference in this parameter among colonies. Medium-size fragments appeared the appropriate size for transplantation as they showed the highest survival. It is also recommended that all sizes of loose colonies should be used for transplantation as attachment increases their chance of survival, which assists natural recovery.

Key words: *Porites lutea*, coral transplantation, coral rehabilitation.

Introduction

For decades, coral reefs in Thailand have suffered from degradation as a result of both natural and anthropogenic disturbances including coastal development, sedimentation, destructive fishing and tourism-related activities. Concerned institutions and organizations from both the government and the private sectors have initiated coral reef rehabilitation projects in an effort to restore the degraded reefs. These projects have been initiated in both the Gulf of Thailand and the Andaman Sea, and implemented with a range of objectives and using different methods (Yeemin et al. 2006). The most common method used in Thailand during the past 10 years has been coral transplantation. Like in many other areas of the world, the coral species used for this purpose were mainly branching corals, i.e. *Acropora* and *Pocillopora*. Branching species recruited well and grew fast (Yap et al. 1992; Bowden-Kerby 1997), but their high mortality rates (Clark and Edwards 1995), their susceptibility to bleaching when stressed, and their susceptibility to disease are significant risks associated with transplantation and reef restoration projects which rely on these species (Edwards and Gomez 2007). By contrast, massive species grow more slowly but they are more resistant to bleaching, and their solid, hard structure enables them to withstand storms and strong waves, thus they are likely to survive better. Few rehabilitation studies have focused on massive coral species due to their slow growth rate and greater difficulty to transplant. However, massive corals are major reef building blocks and many of them are more resilient to natural disturbances, thus more attention should be paid to their use in transplantation.

We chose to study *Porites lutea* transplantation as it is the major reef building species of the Andaman Sea and it has shown some resilience to major natural disturbances such as *Acanthaster planci* outbreaks, increases in sea surface temperature, and storms (Phongsuwan and Chansang 1993). At Maiton Island, unattached or loose *Porites* are common on the sea floor at a depth of 3-5 m. Unless they are attached to a substrate, these corals tend to have a poor chance of survival. In this study, survival and growth of different sizes of both fragments and unattached colonies following transplantation were investigated.

Materials and Methods

Study site

The study was carried out from November 2005 to June 2008 at Maiton Island, 8 km off the southeastern Phuket coastline (Fig. 1). The coral community on the island's northeastern side, where the study took place, lies on a flat sea floor at a depth of about 3-4 m below

mean sea level. In May 1986 the coral reef at Maiton Island and shallow water reefs nearby were severely damaged by a storm and coral recovery has been very low in comparison with reefs nearby (Phongsuwan 1991). The staghorn coral, *Acropora* spp., used to be the dominant genus as shown by debris in the area, but our survey in 2007 on the coral colonies settling on natural substrate found that corals at Maiton Island were dominated by *Porites* and *Millepora* which comprised of 90.48 and 2.69 % of total living corals in the area, respectively. The density of unattached massive coral colonies, including *Porites lutea*, was 1.72 colonies m^{-2}.

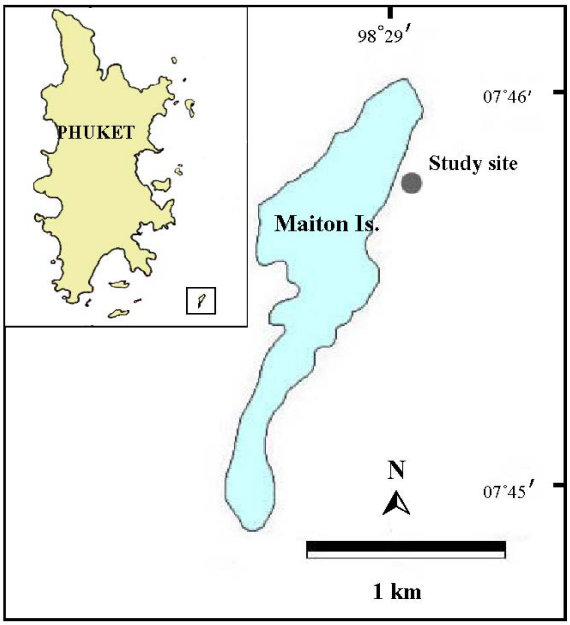

Figure 1: Study site at Maiton Island, Phuket

Transplantation and monitoring

1. Using a hammer and chisel, fragments of *P. lutea* were removed from intact donor colonies and divided into three different sizes, 5.0 x 5.0 cm (large fragment, FL), 3.5 x 3.5 cm (medium-size fragment, FM) and 2.5 x 2.5 cm (small fragment, FS). 45 replicate fragments of each size were generated.
2. Unattached *P. lutea* colonies, which were found on the adjacent seabed, were collected and sorted into 3 size classes as above (small colony, CS; medium-size colony, CM and large colony, CL). 30 replicates of each size were collected.
3. All fragments and unattached colonies were attached to 16 x 28 x 8 cm hollow blocks. Three corals were attached per block using quick drying cement. The blocks with the corals were placed at the same depth as corals were taken.
4. Measurement of the coral transplants was made by scuba divers. Using callipers, the maximum diameter and colony height of the living parts of all colonies were measured every 3-4 months. Survivorship, mortality and detachment of corals from the substrates were also monitored during each survey.

Data analysis

Survivorship, detachment and *in-situ* mortality were calculated as percentages of initial samples under monitoring. Overall survival was defined as the percentage of corals which had suffered neither detachment nor *in-situ* mortality. Growth was measured as both increase in colony area and colony height. Growth of transplanted *P. lutea* in this study predominantly involved horizontal expansion as shown in Fig. 2. Thus colony plan area was calculated by the formula πr^2 with assumption that colonies were circular in shape and r was equal to maximum diameter divided by 2. Regression analysis of growth pattern was done using Microsoft Office Excel 2003. Colony area increased exponentially and colony heights increased linearly. Thus for colony area, the horizontal growth constant (k) for each treatment was calculated using equation:

$$k = (LN(A_t)-LN(A_o))/t$$

where LN is the natural logarithm, A_o is the initial area measurement, A_t is the last area measurement available, and t is the time between measurements in years. Growth rates in terms of colony height increase were expressed as the slope of linear plots

The difference in growth, both in terms of transplant area and height, were compared using ANOVA (SPSS version 15.0). S-N-K tests were employed to determine which treatments differed.

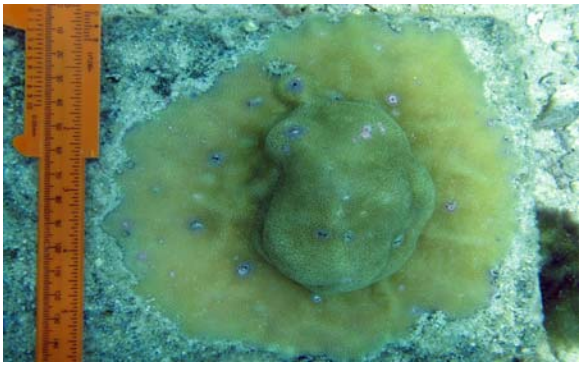
Figure 2: *Porites lutea* colony growing on a hollow cement block two years after transplantation.

Results

The percentage overall survivorship, in-situ mortality and detachment of fragments and colonies after transplantation are shown in Fig. 3. Almost three years after transplantation, the medium-size colonies and fragments, showed the highest survivorship, followed by large fragments, large colonies and small colonies. The small colonies and fragments had very

low survivorship (23.3% and 2.2%, respectively), comprised of a high detachment rate (63.3% and 66.7%, respectively) and in-situ mortality (13.3% and 31.1%, respectively). The high detachment and in-situ mortality occurred 150-300 days after transplantation during the Southwest monsoon season (June-October 2006). Subsequently, detachment and mortality rates of small transplants were similar to other treatments.

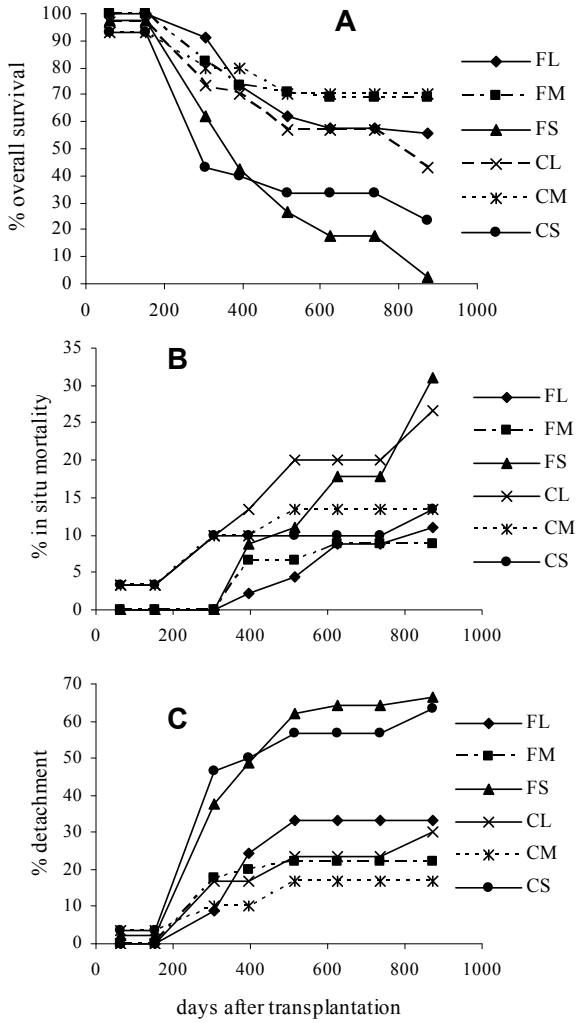

Figure 3: Percentage overall survival (A), *in situ* mortality (B) and detachment (C) of *P. lutea* fragments and colonies after transplantation.

Growth of the transplants

Most corals, both fragments and colonies of all sizes, started to self-attach by growing their basal plate over the cement blocks within two months of transplantation (Fig. 4).

Transplants showed a trend of exponentially increasing area with time (Fig. 5) and a trend of linear increase in height (Fig. 6). Growth rates expressed as increasing area and height per year are shown in Table 1. The growth constant (k) of coral fragments varied from 1.10 yr^{-1} for large fragments to 1.79 yr^{-1} for small fragments, while that of colonies varied from 0.91 yr^{-1} in large colonies to 1.68 yr^{-1} in small colonies. The growth constant of small colonies was significantly higher than that of large colonies (S-N-K, $p = 0.021$).

The colony height began to increase about 6 months after transplantation. Rates of increase in colony height of coral fragments were 0.58, 1.21 and 1.75 cm yr^{-1} in small, medium and large fragments respectively, and were significantly different (S-N-K, $p = 0.024$). Rates of height increase in colonies of different sizes did not differ significantly.

Figure 4: Transplanted colony of *P. lutea* starting to attach to cement block a few weeks after transplantation.

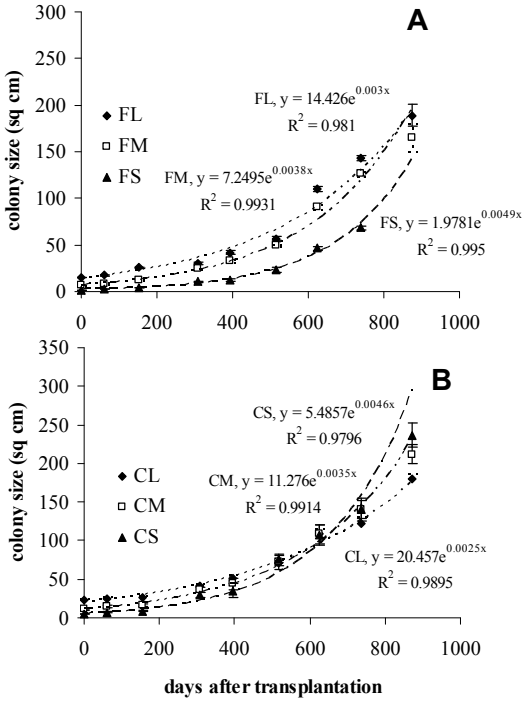

Figure 5: Growth in area of transplanted fragments (A) and colonies (B) with trendlines indicating colony growth.

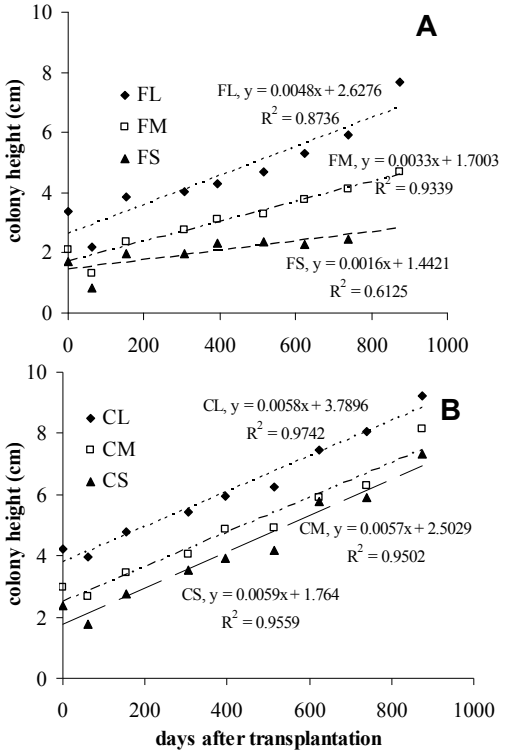

Figure 6: Colony height of transplanted fragments (A) and colonies (B) with trendlines indicating rate of increase of height in cm d^{-1}.

Table 1: Growth rates expressed as growth constant for increase in colony area and annual rate of increase in colony height of transplanted fragments and colonies

Category	Area growth constant, k (yr^{-1})	Height increase (cm yr^{-1})
FL	1.10	1.75
FM	1.39	1.21
FS	1.79	0.58
CL	0.91	2.12
CM	1.28	2.08
CS	1.68	2.15

Discussion

Our study found that it is possible to use fragments and unattached colonies of the massive coral, *P. lutea*, in the rehabilitation of degraded coral reefs. Instead of disturbing donor reefs by using whole large coral colonies, fragments were used. In addition attaching small loose colonies to hard substrata enhances their survivorship which assists natural recovery.

To minimize mortality and detachment of transplanted corals, an appropriate size must be used. Small fragments (2.5 x 2.5 cm) showed high detachment and did not survive well under this study. Although large fragments have a better chance of survival and grow faster than small fragments, breaking too large fragments may put stress on intact donor colonies and affect their growth. Medium-sized fragments grew at similar rates to the large fragments while their chances of survival were higher and their use puts less stress on donor corals than large fragments. Therefore, medium-size *P. lutea* fragments (3.5 x 3.5 cm) appeared most suitable for transplantation at Maiton Island or other areas where the environmental conditions are similar. Likewise, medium-size colonies showed good survival and similar growth rates to the large-size colonies, thus no significant benefit appeared to be gained by using 5 cm as opposed to 3.5 cm colonies.

In a previous study it took at least 7 years for transplanted *Porites lutea* fragments at Maiton Island to cover fully the cement blocks and blend with the natural surroundings. The mean growth of these coral colonies was 2 cm yr^{-1} in height (Thongtham et al. 2003) which is comparable to this study (0.6 to 2.2 cm yr^{-1} in height). Growth of the transplanted colonies at Maiton Island was comparable to growth of *P. lutea* (1.11 to 2.43 cm yr^{-1}) in natural coral reefs at Cape Panwa, Phuket Island (Charuchinda and Chansang 1985).

Acknowledgement

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Predicting reef Futures in the Context of Climate Change: is 500 ppm CO_2 and 2° C of Warming the 'Tipping Point' for Coral Reefs?

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Analysis of reef fish abundance in the Gulf of California, and projection of changes by global warming

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Abstract. The Gulf of California has high endemism and diversity of its reef fish fauna. This study analyzes the abundance of 20 dominant species in the region, and evaluates possible changes to be caused by temperature increments. Stationary censuses of fishes were done in six regions during 2006, and oceanographic information for each one, including mean, minimum and maximum surface temperature, photosynthetic pigments, and nitrate, phosphate and silicate concentrations, was gathered. These factors were included in stepwise regressions to evaluate its influence on individual species, and the equations were used to project change in abundance as a result of warming in accordance to three scenarios: +1°, +2° and +3°C. The models indicated that as temperature rises, 4 species may reduce their abundance, 14 increase it and 2 may remain stable; also, 6 species may extend their geographic ranges. At community level, richness, taxonomic distinctness, diversity and average trophic level will likely increase with temperature, but the latter two will eventually return to their original levels. In conclusion, the Gulf of California fish fauna will not react homogeneously to the warming; some species may change their distribution and shifts in composition and structure are expected. These events may cause an ecological imbalance in teleost assemblages, and consequently affect the functions of the reef ecosystem in the gulf.

Key words: Temperature increase, distribution, community structure.

Introduction

The Gulf of California is recognized by its high endemism, diversity and biomass (Roberts et al. 2002, Mora and Robertson 2005), and consequently this inner sea represents one of the main areas for reef fisheries in México (SAGARPA, 2000). The Gulf is home to about 800 fish species, 271 of which are resident of rocky and coral reefs (Thomson et al. 2000), and they play a key role in these systems because their abundance and biomass makes them key elements in local trophic webs, and in the energy flow between neighboring biomes (Mumby et al. 2007).

Studies of the Intergovernmental Panel of Climate Change mention that in the last 30 years the atmospheric temperature increased between 0.3° and 0.6°C, and different models show that it will rise between 1.4° and 5.5°C by the end of the century (IPCC 2007). The panel also suggests that each of 1°C atmospheric increase, will lead to an 0.5° increase in the ocean. Many have indicated that reef faunas will be much affected by warming (Roessig et al. 2004; Perry et al. 2005; McKenzie et al. 2007). Fishes can be particularly disturbed by higher temperatures because they are ectotherms and mobile, and thus modifications in their occurrence and abundance can be rapidly detected (Bellwood et al. 2006).

The increasing trend of sea temperature worldwide is not homogeneous, as local and regional conditions have a definite influence (IPCC 2007; Hayes and Goreau 2008). However, it is important to generate scenarios in order to predict the potential impact of warming in local ecosystems, and from there provide recommendations to managers and government officials. Under this perspective it is especially relevant to analyze the current distribution, abundance and richness of marine resources exploited by man, and to plan ahead; this can support preservation of the assemblages and sustainable development. This paper presents an analysis of the current state of the abundance of 20 common fishes from the Gulf of California; we prepared numerical models for each species to predict changes in abundance and distribution under different levels of temperature increase. The results indicate that each species will behave differently and thus it is not expected that the fish assemblages in the Gulf of California will change in unison in the near future. Because of this idiosyncratic situation, it is possible that that global change will eventually have a strong effect on the function of the reef fish faunas, even when their composition may not change that much.

Material and Methods

The work was conducted in The Gulf of California, México. It is an inner sea adjacent to the Pacific Ocean, with a mixture of tropical and sub tropical waters and considerable differences in temperature between the north (average ~22°C, range 14° to 31°C) and south (average ~25°C, range 18° to 30°. Thomson et al. 2000). Stationary census (N=147) were done in six regions (Fig. 1); Los Angeles Bay (28°N), Santa Rosalía (27°N), Loreto (26°N), La Paz Bay (24°N), Cabo Pulmo (23°N) and Los Cabos (22°N). Fish abundance was estimated by counting fishes moving across observations cylinders of 5 m radius (79 m^2 sampling area), in a time period of 15 minutes. Of this interval, 5 minutes were dedicated to register species, and the following 10 to count individuals (Villarreal-Cavazos et al. 2000). The censuses were conducted in 2006, at a depth range between 5 m and 15 m, where reef fish abundance is higher (Alvarez-Filip et al. 2005; Robertson and Allen 2006). The studied regions present predominantly rocky bottoms with low coral cover (ranging from 20% at Cabo Pulmo, to 2% or less from Loreto to Los Angeles Bay; Reyes Bonilla and López Pérez, in press).

Each surveyed site was referred to a single 1° x 1° latitude-longitude cell, and for each of the six quadrats we obtained the mean, minimum and maximum surface sea temperature (°C), from the Reynolds SST analysis (1982 to 2006; www.nhc.noaa.gov/aboutsst.shtml), average photosynthetic pigments concentration (mg/m^3) from the SeaWifs and MODIS satellites (www.science.oregonstate.edu/ocean.productivity/custom.php; 1997 to 2007), and the mean nutrient concentration (nitrate, phosphate and silicate; μM), from the World Ocean Atlas 2005 (http://www.nodc.noaa.gov/OC5/SELECT/woaselect/woaselect.html), from 1950 to 2004.

Before conducting the numerical analysis, we selected the 20 most abundant species of the full database which included over 80 taxa seen in the six visited regions, These 20 species belong to six families and as they occupy different reef habitats and present a variety of morphologies, food sources and life histories, we considered them as good representatives of the entire fish community. To generate models for prediction of abundance of each fish species in accordance with environmental factors, we used stepwise regressions; this technique also allowed us to quantify the influence of each oceanographic indicator on abundance. All data were standardized, and to avoid autocorrelations the regressions were run with the "ridge" routine, using a tolerance of 0.00001 (Neter et al. 1996).

The next step was to evaluate the possible impact of ocean warming on the targeted fish species, using three possible scenarios considered by the IPCC: increment of 1, 2 and 3°C. To do so, we directly replaced the value of the new coefficient of temperature in the regression equations, when this attribute was a part of the model. In addition, the coefficients of the remainder oceanographic features were adjusted correspondingly with the proposed warming by using the estimated value obtained from linear regressions of temperature and each factor, in order to predict its response to elevation of 1° to 3°C. With these corrections the final models were more complete and able to predict the joint effect of several environmental factors at the same time. When temperature was not selected by the stepwise procedure, we only used the modified parameters in the models of fish abundance.

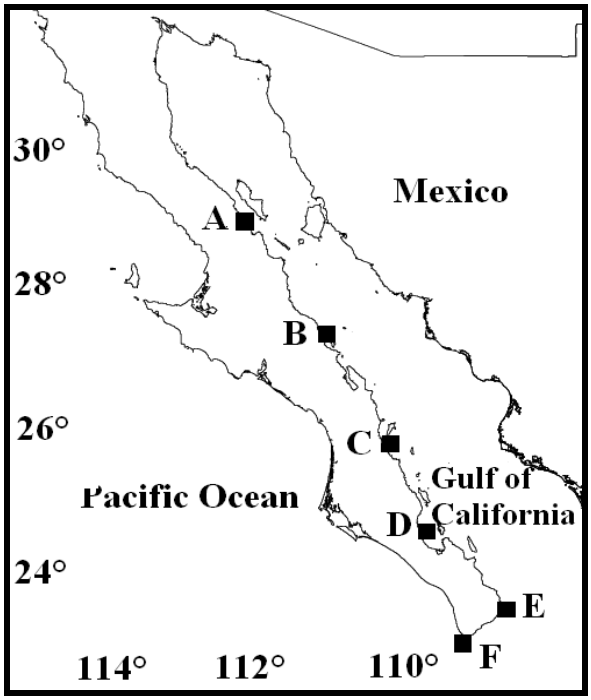

Figure 1: Study regions in the Gulf of California, Mexico; A) Los Angeles Bay, B) Santa Rosalía, C) Loreto, D) La Paz, E) Cabo Pulmo and F) Los Cabos.

Finally, we tested the consequences of changes in individual species at a higher hierarchical level by generating "future" communities from the predicted abundance of the 20 species. Then, for all regions and scenarios we calculated four ecological indices: richness, Shannon-Wiener diversity, taxonomic distinctness, and the similarity of the predicted communities to the actual data using the Bray-Curtis coefficient, and performed a cluster analysis, comparing all regions and scenarios.

Results

There were four different types of response in the 20 studied taxa. First, the models suggest that four species could reduce their abundance, including the now widespread damselfish *Abudefduf troschelli*. It is relevant to notice that the predictions point out to the eventual disappearance of a Gulf of California endemic *Chromis limbaughi*, the blue-yellow damselfish, in the northern gulf (Fig. 2).

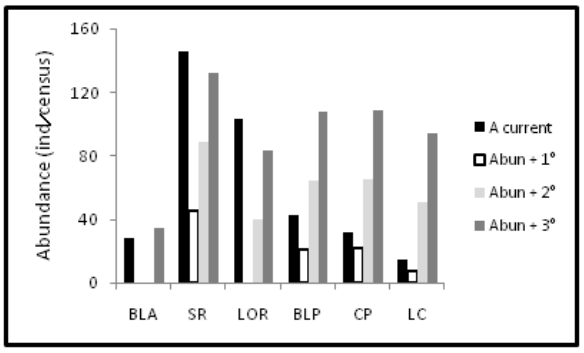

Figure 2. Current and predicted abundance of *Chromis limbaughi*.under three different scenarios of temperature increment

Other 13 species could increase their abundance and in some cases their numbers may become very similar in all areas of the Gulf (Table 1). One of them is very important for the aquarium trade (the Cortes angelfish *Holacanthus passer*), and among the rest there are two very common fishes: *Chromis atrilobata* (spot damselfish) and *Thalassoma lucasanum*, (the rainbow wrasse), as well as a key commercial species, the yellow snapper *Lutjanus argentiventris*. Finally, two species (a haemulid and a snapper) show no change caused directly or indirectly by the warming, possibly indicating a wide physiological tolerance.

Table 1 Predictions of change in abundance and geographical range of the 20 analyzed species, with temperature increases.

	Abundance		
Range	**Increase**	**Decrease**	**Same**
Increase	*P. punctatus* *C. puctactissima* *S. flavilatus* *C. oxycephalus* *S. ghobban* *T. grammaticum*		
Decrease		*A. troschelli* *G. simplicidens* *C. gracilis* *C. limbaughi*	
Same	*M. dentatus* *H. passer* *B. diplotaenia* *C. atrilobata* *T. lucasanum* *S. rectrifaenum* *L. argentiventris* *H. flaviguttatum*		*P. colonus* *H. maculicauda*

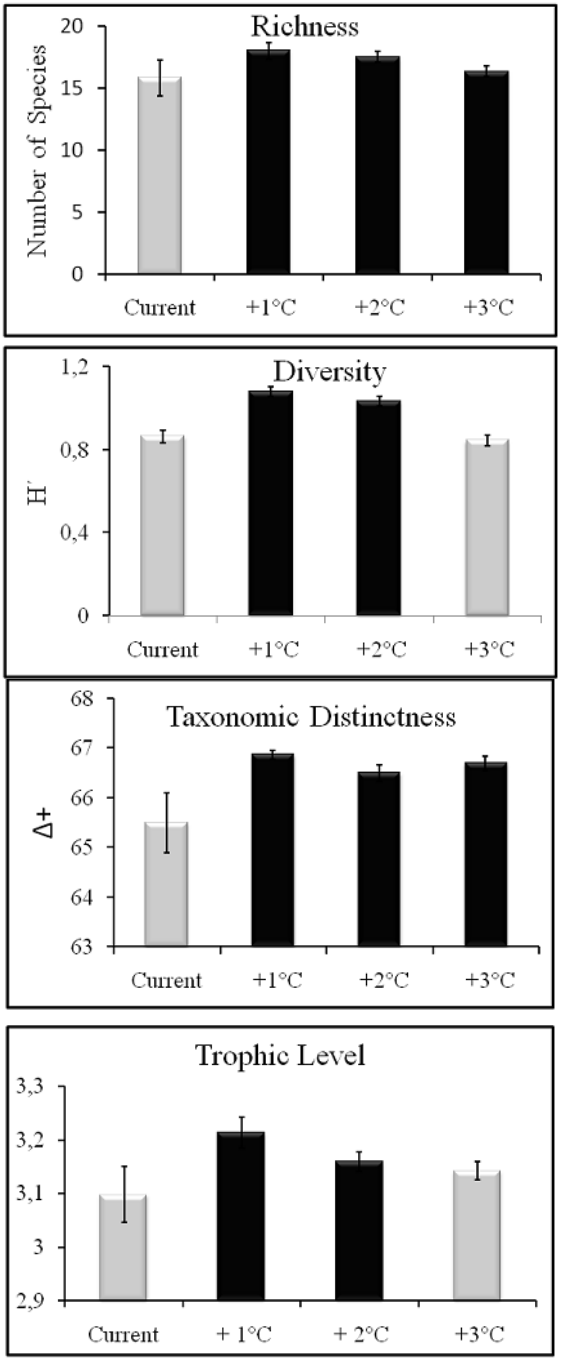

Figure 3. Changes in fish community structure according to three possible scenarios of global warming. Bars signal the average and SE, and those with the same color had no significant differences, according to Kruskal-Wallis tests.

Related to changes in distribution, six species could extend their ranges to areas where they are currently absent, in specific Los Angeles Bay. In this case we have a commercial species (*Scarus ghobban*) and a very important herbivore *(Prionurus punctatus*).

Another four species will likely have a more restricted range, and 10 apparently will be unaffected (Table 1).

The community analyses evidenced that richness, diversity, taxonomic distinctness and trophic level, significantly increase when the temperature rises 1°C. However, their values gradually lower, and in the case of diversity and trophic level at +3°C, they even return to approximately the same level they were before temperature changed (Fig. 3). Finally, the dendrogram (Fig. 4) shows that the fish communities will change depending on the temperature increase from their original condition (left side of the tree), to +3° (right side). Notwithstanding there will be a mix of assemblages since conditions starts to change, meaning that the compositional shift will not be completely ordered.

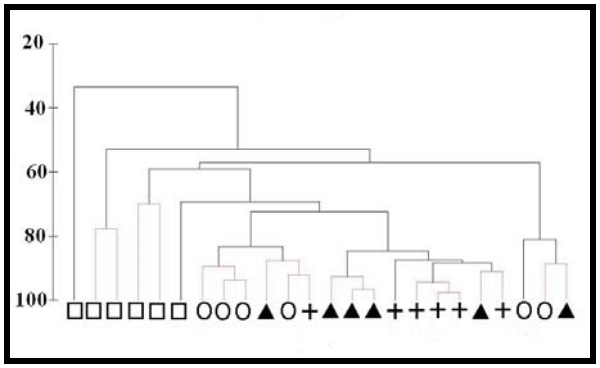

Figure 4. Dendogram showing the similarity in the current and the projected assemblages. Key: squares= actual assemblage, circles= +1°C, triangles= + 2°C; crosses= + 3°C.

Discussion

The model tested in this paper considers the response of 20 fish species to potential changes in a set of oceanographic variables including productivity and nutrient concentration; nevertheless, the forecasting is ultimately based on how sea surface temperature will increase in the Gulf of California. There are several reasons why this choice was made; first, global change models only consider sea temperature and salinity as there are excellent long-term data on the former factor, and the second can be predicted accurately from the first (IPCC, 2007). In addition (as it was done in this paper), the relation between temperature and other chemical forms in the ocean can be locally or regionally modeled (Kamykowski 2008; Silió-Calzada et al. 2008) but the link breaks at a global scale. Consequently, Donner et al. (2009) recommend that analyses of the response of reef faunas to global change should focus on temperature increase, and this justifies why most studies on the future of reef corals have also followed this approach (Sheppard 2003; Donner et al. 2005).

There are other variables which may affect the response of reef fishes to ocean warming (Baker et al. 2008; McClanahan et al. 2009). They include changes in habitat quality (specifically loss of coral cover and reef structure), in ocean circulation (which will affect dispersal and recruitment), and in acidity. Considering the first topic, reef coral cover is low along the Gulf of California (Reyes-Bonilla and López-Pérez, in press). As a consequence, most reef fish species of the Gulf of California do not depend on the presence of corals for their settlement, feeding or protection, as shown by their community stability even after severe bleaching and mortality events (Alvarez-Filip et al. 2006). For that reason we suggest that the ocean warming will not affect significantly the condition of the local fish habitats, and thus the factor was not examined here.

In relation to ocean acidity, there are worries that a lower pH can affect metabolism, larval development and other aspects of the life history of the ichtyofauna (Baker et al. 2008). Unfortunately the situation is still not clear, and Munday et al (2008) stated that any kind of prediction is still speculative as the experimental and field evidence is scant. Something similar can be said about ocean currents as there are contrasting views on the subject (IPCC 2007). Considering this, we decided not to include any of these factors in the regressions.

One of the key findings of this study is that the reef fishes of the Gulf of California will not react homogeneously to temperature increases. This was expected since the fish assemblages of the Gulf are formed by a mixture of tropical and temperate faunas (Thomson et al. 2000), with different niches and ecological requirements, and which will adjust differentially (or not at all) to oceanographic changes. Table 1 indicates that the most affected species (with reduced abundance and distribution range) are adapted to warm or cold water (*Abudefduf troschelli* and *Girella simplicidens*); probably the former will disappear because their upper temperature tolerance levels are reached, and the latter if the Gulf becomes too warm (Roessig et al. 2004; Perry et al. 2005). However, the models indicate that most species will benefits from the warming by either increasing their geographic range or their local population sizes (Table 1). A good explanation for this situation is that as practically all of the studied taxa are distributed in the Panamic Province, they are adapted to tropical conditions (Fiedler and Talley 2006) and then the ocean warming will provide them a more adequate habitat in the Gulf of California.

It was interesting that the ecological indices reach higher values with temperature increases (Fig. 3). This possibly results from the concurrent rise in richness and abundance of the fishes (especially in the

northern Gulf), but the change is not "permanent" as diversity and average trophic level return to the values of 2006. This finding points out that the effects of global change may be subtler than imagined, and that as proposed by Bellwood et al. (2006), traditional ecological indices may not be good indicators of effects on ecosystem condition. However, note that taxonomic distinctness remained high, indicating a more homogeneous distribution of abundance and species richness in higher taxonomic groups in the future. This metric is recognized as a good indicator of biodiversity (Magurran 2004), and its response in this paper indicated that it can be a good choice to depict future changes in the fish faunas.

According to the dendrogram (Fig. 4), the compositional changes caused by higher temperature may be relatively orderly, but nevertheless the shift in ecological function of the resident species (shown by the average trophic level; Fig. 3), point out to possible ecological imbalances in reef fish assemblages of the Gulf in following decades; these in turn may translate to potential economic impacts. A similar statement has been done in relation to previous modifications in fish abundances along the Gulf, in particular the depletion of larger carnivores (Sala et al. 2004). The combination of unsustainable fisheries and global change may bring a bleak future for reefs in this region.

In conclusion, our results show that fish species of the Gulf of California might respond differently to increases in ocean temperature and thus the communities will not change as a unit. Because of these qualitative changes, global warming might have severe effects on the function and productivity of the local reef ecosystem.

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Does body colouration influence predation risk of coral-dwelling reef fish in bleached landscapes?

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Abstract: Coral reef fishes will be increasingly exposed to habitat degradation as climate change increases the frequency and intensity of coral bleaching events. This study explores how body colouration influences survivorship of coral-dwelling fishes during coral bleaching events. Specifically, we tested for differences in the vulnerability to predation between a bright yellow fish (*Pomacentrus moluccensis*) and a white/black fish (*Dascyllus aruanus*) in a range of different coral habitats, including healthy, bleached, recently dead and algal-covered coral colonies. Strike rates by predators against both damselfish species increased in bleached and dead coral habitats, compared to healthy coral habitats. There was no differences in strike rate towards either species of fish in any of the habitat conditions tested, suggesting that both the yellow and black/white colouration is equally visible to the predator (*Pseudochromis fuscus*). However, survivorship on bleached corals was lower for *D. aruanus* than for *P. moluccensis* suggesting that coral bleaching may have a disproportionate effect on brightly coloured reef fishes.

Key words: Climate change, Coral bleaching, Reef fishes, Habitat degradation, Selective predation

Introduction

Corals are essential for the survival of many species of coral reef fishes as they are the primary habitat-forming species in coral-reef environments (Connell et al. 1997). Live coral is a source of shelter, food and recruitment for many coral reef fishes (Pratchett et al. 2008, Jones et al. 2004) while increased topographic complexity moderates competition and predation (Beukers and Jones 1997, Holbrook and Schmitt 2002, Almany 2004). Coral cover and diversity can positively influence the abundance and diversity of coral reef fishes (Bell and Galzin 1984, Carpenter et al. 1981, Munday et al. 1997, Ohman and Rahajasura 1998) and changes in the condition or abundance of coral reef habitat may have considerable consequences for biotic interactions, abundance and diversity of reef fish assemblages (Syms and Jones 2000, Munday 2004, Pratchett et al. 2004, Wilson et al. 2006).

The pervasive view in literature investigating effects of climate-induced bleaching on fishes is that populations decline as a result of a loss in habitat structural complexity following bleaching induced coral loss (Lewis 1997, Bergman et al. 2000), while coral mortality *per se* has limited effects on fishes (Sale 1991, Lindahal et al. 2001). However, several recent studies suggest that mere bleaching of corals can have a detrimental effect on coral reef fishes, especially among those species that inhabit live corals (Ohman et al. 1999, Jones et al. 2004). Decline in the abundance of coral-dwelling fishes following host coral bleaching may be attributable to either movement of fishes to alternative habitat colonies, or mortality, which probably results from increased susceptibility to predation (Wilson et al. 2006, Pratchett et al. 2008). Coral-dwelling fishes may face an immediate increase in susceptibility to predation due to the increased conspicuousness of prey fishes against the white background of bleached corals.

Increased levels of predation could possibly be influenced by a reduction in camouflage of brightly coloured reef fish against the now pale/white habitat background. Different coloured fish associated with habitats exhibiting different levels of pigmentation are therefore predicted to experience a difference in their visibility to predators. Most coral-dwelling fishes are vividly coloured with yellow, blue, green and red which are important in communication and signalling (Lorenz 1962) and can be an effective camouflage against coral habitats (Cott 1940, Marshall 2000). It seems intuitive that brightly coloured fishes should be much more apparent against a bleached-white background, compared to the pink or brown hues of live coral. This contrast between coral-dwelling fishes and pale/white bleached coral is predicted to increase the risk of predation for

brightly coloured fishes as they contrast against the degraded background.

The aim of this study was to compare predation risk of two coral-dwelling damselfish with highly contrasting colouration; one that is bright yellow: *P. moluccensis*, and one that is black and white: *D. aruanus*. The two contrasting fishes were presented to predators against different habitat backgrounds to test if there was a change in the visual detection of prey by a common predator when associated with healthy, bleached, dead and algal-covered coral habitats. Survivorship was also measured across the four different habitats to test the efficiency of the predators at capturing the two different coloured prey. Marshall (2000) found that the yellow coloration in fishes were a very close match to average reef colour with a colour distance of 0.04. Fishes also lack long wavelength photo receptors (Marshall 2000) meaning that yellow will be well camouflaged against an unbleached reef backdrop but will render them more obvious to predators against bleached white coral. The bright yellow colouration of *P. moluccensis* was expected to be more obvious to the predator against the bleached and dead background than the white/black colouration of *D. aruanus* and therefore *P. moluccensis* was expected to elicit a higher number of strikes and have lower survivorship in bleached habitat.

Materials and Methods

This study was conducted in April 2007, at Lizard Island Research Station (14°40'S, 145°28'E), on the northern Great Barrier Reef (GBR), Australia. Aquaria-based experiments were used to test the apparent conspicuousness of prey fish and compare predation risk in different habitat conditions. Coral habitat used in the experiments was *Pocillopora damicornis*, a common branching coral that is highly susceptible to bleaching (Marshall and Baird 2000) and used by many coral-dwelling fishes for habitat (Pratchett et al. 2004). Healthy and algal-covered colonies of similar size and complexity were collected from the lagoonal area. One third of the healthy colonies were randomly selected and subjected to osmotic stress (100% freshwater for 5 mins) to induce bleaching (Kerswell and Jones 2003). This was signified by a reduction of pigmentation within the live coral tissue. Another third were placed into a chlorine solution and rinsed well to remove all living tissue leaving bare white skeleton. Healthy and algal-covered colonies were kept in high flow-through tanks to ensure that they did not degrade.

Juvenile *Pomacentrus moluccensis* and *Dascyllus aruanus* (12-32mm in total length) were used in experiments. Both these planktivores associate with live branching coral and take refuge within the branches of their host coral colony when threatened (Allen 1991). *Pseudochromis fuscus* (53-72mm in total length), was selected as the predator to use in experiments because it a common resident predator on small coral-reef fishes and amenable to experimentation and to aquarium conditions (McCormick and Holmes 2006). Both prey fish and predators were collected from reefs within the Lizard Island lagoon using the anesthetic clove oil and small hand nets. Prey fish were kept in groups of up to ten individuals and fed daily using manufactured aquarium food. Predators were kept in separate aquaria due to their aggressive nature and starved a minimum of 2 days prior to each experiment. Each fish was used only once per trial for each experiment.

To test if the visual detection of two different coloured fish by a common predator varied against different backgrounds, individual juvenile *P. moluccensis* and *D. aruanus* were placed before life-sized laminated photos of i) healthy, ii) bleached, iii) dead and iv) algal-covered colonies. The use of photographic images was used to help eliminate variation in habitat complexity and olfactory stimuli that might influence predatory behaviour. The four pictures were fixed equal distance from each other around the periphery of a large circular plastic tub (113cm dia.). The bottom of the tub was covered in a thin layer of sand and the water level adjusted to the top of the pictures so that the predator remained at eye level to the image. An individual prey fish was placed within a small clear zip-lock bag (50 x 100mm), flooded and placed in front of an image. A predator was released onto a small fragment of coral in the centre of the aquarium. Each trial ran for 20 minutes and the number of strikes against prey in front of each habitat image during this time was recorded. Only one species of prey was used for each trial and each individual prey and predator was replaced after the completion of a trial.

If live, healthy coral provides effective camouflage for brightly coloured reef fishes, such as *P. moluccensis*, we predicted that strike rates by predators would be lower for prey placed in front of images of healthy colonies compared to bleached or dead coral colonies. We also predicted that strike rates would be higher for the bright yellow coloured *P. moluccensis* compared with *D. aruanus*. The black and white colouration of *D. aruanus* might be expected to offer better camouflage against bleached corals than the bright yellow colouration of *P. moluccensis*. Variation in strike rates between the two coral-dwelling fish

was tested in a MANOVA, as recommended by Roa (1992) because individual strikes by predators might not be independent of each other.

Secondly, tests of survivorship with varying habitat condition were conducted to test predation rates. Here prey fishes were subject to predation by predators by placing four prey fish and one predator within an aquarium with one of the four different habitat treatments. Two individual *P. moluccensis* and two individual *D. aruanus* were placed within a glass aquarium (60 x 30 x 40cm) and survivorship recorded over a 75 hour period with the presence of a single *P. fuscus*. Each habitat treatment was replicated six times and survivorship compared. Two tanks were also run with the absence of a predator to control for tank and habitat effects on survivorship. Predators were placed into the tank at 17:00, one hour after the prey fish to allow them to acclimate to the tank and surrounding habitat. Variation in prey survivorship over 75 hours was tested in a MANOVA.

Results

The number of strikes against yellow versus white/black fish did not differ across the four different habitat conditions (MANOVA $F_{4,23}$=0.95, P=0.45). The number of strikes against both fish colours increased when the fish were presented against a background of bleached coral but decreased against backgrounds that depicted algal-covered coral (Fig.1). This suggests that there is no difference between the levels of visual perception between the two fish, even when placed before different habitat conditions. However, overall there was an increase in the number of strikes from healthy to bleached and to dead coral habitat, showing an increase in strike rates with coral degradation.

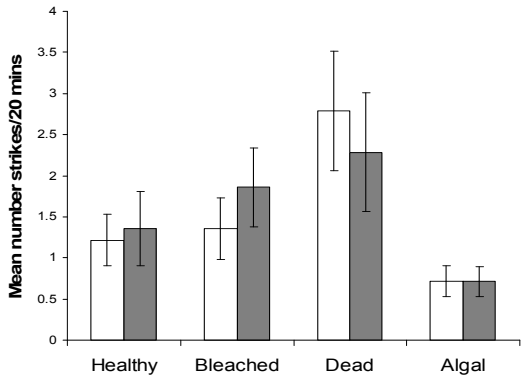

Figure 1. Mean number of strikes (±SE) against *D. aruanus* (□) and *P. moluccensis* (■) associated with four different habitat conditions over a 20 minute period.

In actual predation trials, survivorship between yellow and white/black fish was only significantly different for fish associated with bleached habitat (MANOVA F=7.10, df=1, P=0.04). In bleached coral habitats survivorship was twice as high for the white/black coral-dwelling fish (*D. aruanus*) compared to the yellow species (*P. moluccensis*) (Fig. 2). This suggests that the colour of prey fishes against their coral habitat is critical to evading predators and maximising survivorship.

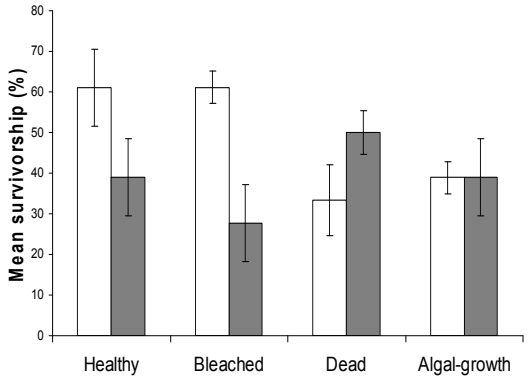

Figure 2. Mean survivorship (±SE) of *D. aruanus* (□) and *P. moluccensis* (■) associated with four different habitat conditions after being exposed to a predator for 75 hours.

Discussion

Predation is a fundamental process on coral reefs influencing the structure of ecological communities (Caley and St John 1996, Hixon and Carr 1997, Almany 2003). The effect that predators have on fish populations is largely driven by the method in which they select prey species and individuals (Hixon 1986). Furthermore, habitat complexity and habitat quality can certainly moderate the outcome of predator-prey interactions (Main 1987). This study explored if two different coloured fish vary in susceptibility to predation across a range of habitat conditions given that one species (*P. moluccensis*) is bright yellow and the other (*D. aruanus*) is white and black. We found that there was no significant difference in strike rates on these two contrasting prey fishes against a range of different background habitats and importantly, an increase with coral degradation for both fish colourations. Similarly, the survivorship of the two different coloured prey fish was broadly similar over a range of habitat conditions, except when the coral was bleached. In bleached coral habitats, it was clear that *D. aruanus* had a decided advantage in survivorship over *P. moluccensis*, possibly because bleached coral habitat continues to provide an appropriate background to minimise conspicuousness of white and black fishes.

Different predation rates on reef fishes associated with healthy, bleached, dead or algal-covered coral habitats is possibly a result of increased visual perception by predators. Many reef fishes are specifically coloured to camouflage against their habitat for hunting, hiding and signalling (Cott 1940, Marshall 2000). Camouflage is an important determinant in the distribution and habitat associations of many animals by matching body colouration with their surrounding habitats (Helfman et al. 1997). The similar strike rates on the two species of fish suggest that there was no difference in the visual detection by a predator of the yellow versus the white/black body colouration. Although the white/black colouration of *D. aruanus* might be expected to visually stand out against healthy and algal-covered coral habitats, there was no significant difference in the number of strikes observed compared with *P. moluccensis*. Both damselfishes received more strikes as the habitat degraded from healthy to bleached to dead colonies, although fish associated with algal-covered coral had the lowest number of strikes. Declines in the abundance of *P. moluccensis* immediately following coral bleaching have been repeatedly documented along with the declines in other coral-dwelling fishes (Booth and Beretta 2002, Pratchett et al. 2008). This is consistent with the expected visual contrast between fish and habitat. The low number of strikes for both prey colours on algal-covered coral might suggest that this dark brown/green habitat offers the best backdrop for the fish to camouflage against or that predators are used to looking for prey in healthy and similar looking habitats.

Survivorship between the two prey colourations was similar across the different habitat conditions except for bleached corals where mortality was slightly lower or *D. aruanus*. *Dascyllus aruanus* was expected to have better camouflage against the bleached habitat as the white background would have a reflectance similar to that of the fish; white fishes reflectance peaks between 330 and 390 nm while the black part has very low reflectance (Marshall 2000). This contrast between white and black could potentially provide disruptive camouflage that could confuse the predator against the bleached white background. Therefore, contrasting colour patterns as seen in *D. aruanus* could be just as important for predator avoidance as colour. The white and black contrasts could break up the body outline and confuse predators.

In conclusion, this study shows that bleaching is likely to have a disproportionate effect on brightly coloured fishes provided predation is the main proximal cause of mortality, and that colour vision plays a role in prey detection and predator avoidance through camouflage. While the predatory species used in this study (*P. fuscus*) is a conspicuous predator within coral habitats occupied by coral-dwelling damselfishes (Munday et al. 2003), it should be noted that other predators on coral-dwelling fishes may detect prey fishes at different distances or using other cues which may not be influenced by visual changes to habitat condition. Further research is needed to investigate how changes in habitat condition, through loss of pigmentation, live tissue cover and natural habitat structure, will impact the ability of coral-dwelling fishes to avoid predators. Colour could be one method of camouflage. Contrasting colour patterns may also be effective in reducing predation risk, especially in bleached coral habitat.

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Coral reefs of the Gulf of Mannar, Southeastern India - observations on the effect of elevated SST during 2005-2008

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Abstract. The effect of elevated sea surface temperature (SST) on the coral reefs of the Gulf of Mannar, Southeastern India was monitored during 2005-2008 using quadrate and LIT methods. The pattern of effect was almost similar on the reefs every year except the modest fluctuations in the temperature levels. The temperature varied between 31.0^{0}C and 33.5^{0}C during summer (April - June). The major reef areas in the Gulf of Mannar are shallow, between 0.5-3.0 m depth and in general, comparatively high temperature prevailed and the reefs seem to be acclimatized to such situation. The average percentage of bleached corals during 2005, 2006, 2007 and 2008 was 14.6, 15.6, 12.9 and 10.5 respectively. The bleaching of corals was noticed from mid April and high temperature existed for about a month from the end of April. Massive corals especially *Porites* sp. were the first to be affected and the other dominant coral species partially / fully bleached were *Acropora cytherea, A. formosa*, *A. intermedia, A. nobilis, Montipora foliosa*, *M. digitata* and *Pocillopora damicornis*. The incidence of bleaching was not uniform every year, in terms of area and depth, but the pattern was same. Dependent on rainfall and winds, recovery began during June-July and completed in 1-4 months. The branching corals recovered quickly after temperature reduction, but massive corals took longer time. The fastest recovered coral size groups were 40-80 cm and 80-160 cm. There was no coral mortality in 3 years due to elevated SST, but 80% of the bleached recruits dead in 2007.

Key words: Coral reef, climate change, annual bleaching, SST

Introduction

Coral reefs appear to be undergoing worldwide decline (Epstein et al. 2001; 2003). An estimated 20% of coral reefs worldwide have been destroyed, while 24% are in imminent danger and a further 26% are under longer term danger of collapse (Wilkinson 2004). Although much of the coral reefs degradation is directly blamed on human impact, there are natural disturbances causing significant damage to coral reefs.Global warming is undoubtedly important, many instances of bleaching being correlated with increased temperature (Glynn 1991; Glynn 1993; Warner et al. 1999). Increased sea surface temperatures, decreased sea level and increased salinity from altered rainfall can all result from weather patterns such as El Nino (Forrester 1997). Unprecedented anthropogenic pressures on reefs have been exacerbated in the 1980s and 1990s by several strong El Nino Southern Oscillation events, which have been correlated with widespread warm water anomalies and associated bleaching and mortality of corals (Glynn 1984; 1993; Wilkinson et al. 1999).

Reefs in the Gulf of Mannar are developed around the 21 uninhabited islands that lie along the 140 km stretch between Tuticorin and Rameswaram of Tamilnadu, Southeast coast of India (Fig.1).

Figure 1 Map showing Gulf of Mannar

These islands are located between latitude 8º47' N and 9º15'N and longitude 78º12'E and 79º14'E and the average distance of these islands from mainland is about 8 km. The once rich reefs of Gulf of Mannar have been heavily exploited in the past 2-3 decades. Now, due to conservation initiatives, tightening of the enforcement and permanent halt of the coral mining, coral cover is increasing remarkably. However, significant coral bleaching was observed in the Gulf of Mannar in 2005. The present monitoring was initiated to collect baseline information on temperature variation and its impact on corals.

Materials and methods

The temperature was measured monthly in the reef areas of all the islands of the Gulf of Mannar during the period between 2005 and 2008 by digital thermometer. Permanent sites at each islands assessed the bleaching. Percentages bleaching during the summer months and recovery after consequent months were estimated using Line Intercept Transect method (English et al., 1997) and quadrate method.

Results

Temperatures in the Gulf of Mannar are never below 26 ºC. Summer temperatures (April to June) varied between 31.0º C to 33.5ºC. Sea surface temperature peaks during May (overall range 26.5 to 30.5ºC; Fig.2-3).

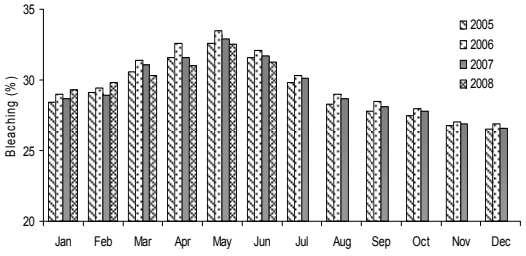

Figure.2: Average monthly SST values in the Gulf of Mannar during 2005 to 2008

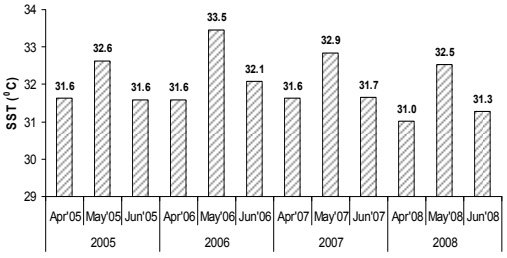

Figure 3: Sea surface temperature (^{0} C) in summer (April, May and June) during 2005 to 2008 in the Gulf of Mannar

Corals bleached every year since 2005 during summer starting from April. Average percentage of bleached corals was 15.6% during 2006, 14.6% in 2005, 12.9% in 2007 and 10.5% in 2008 (Fig.4).

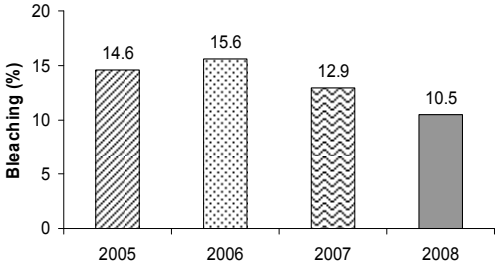

Figure 4: Percent of bleaching in summer (April, May and June) during 2005 to 2008 in the Gulf of Mannar

Massive corals especially *Porites* sp. were the first to be affected and the other dominant coral species partially or fully bleached were *Acropora cytherea*, *A. formosa, A. intermedia, A. nobilis, M. foliosa, M. digitata* and *Pocillopora damicornis*. *A. cytherea* bleached the most (2.91% during 2006) followed by *A. formosa* with 2.55%. In 2005 the most affected species was *A. cytherea* with 2.44% followed by *M. digitata* with 2.12%. In 2007, the highest affected was *Porites* sp. with 2.05% followed by *A. cytherea* and *A. intermedia* and in 2008 it was *Porites* sp. with 1.75% followed by *A. formosa* with 1.66%. The details are given in the Fig.5.

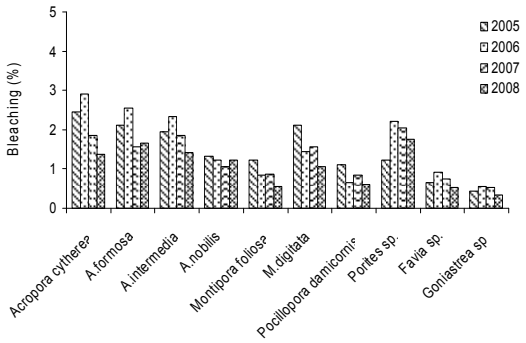

Figure 5: Percent of bleaching in common coral species in the Gulf of Mannar

Incidence of bleaching was not uniform every year in terms of area and depth, but the pattern was comparable. Depending on rainfall and winds, recovery began during June-July and was completed in 1-4 months. The branched corals recovered quickly after temperature reduction, but massive corals recovered slower.

The fastest recovered coral size groups were 40-80 cm and 80-160 cm. There was no coral

mortality in 4 years due to elevated SST, but 80% of the bleached recruits dead in 2007.

Discussion

The projected increases in ocean temperatures are expected to exacerbate the stressors already affecting many coral reefs, resulting in additional coral bleaching and mortality (Pockley 2000; Hughes et al, 2003; Pandolfi et al. 2003). However, the reported annual bleaching in the Gulf of Mannar did not result in any appreciable mortality, maybe because the area has already been severely disturbed. In the Gulf of Mannar, corals tend to bleach when exposed to 2 to 3ºC elevated temperature level in late April every year. Hoegh-Guldberg (1999) predicted that mass bleaching could become an annual occurrence by 2020 in Southeast Asia and the Caribbean, by 2030 on the Great Barrier Reef and by 2040 in the central Pacific. This prediction is already reality in the Gulf of Mannar since the bleaching event happens every year in the same time since 2005.

Corals that are regularly exposed to stressful environmental conditions have, in some cases, been shown to acclimatize and exhibit physiological tolerance to elevated temperatures and UV-radiation that exceed normal thresholds. Reef areas in the Gulf of Mannar are shallow, (0.5-3 m depths), and high temperature around 29ºC prevails throughout the year. Corals seem to be acclimatized but temperature over 31.0ºC causes bleaching and the corals start to recover when the temperature level falls to normal.

Factors influencing resistance of corals to high temperatures include extrinsic (environmental) factors such as strong water currents that reduce the severity of thermal stress (West and Salm 2003; Jokiel 2004), and intrinsic (physiological) factors such as incorporation of heat-resistant zooxanthellae clades (Baker 2004) and the production of heat shock proteins (Brown et al. 2002). Identifying and protecting habitats and colonies that are relatively resistant to high temperatures is important for conserving coral reefs in the face of global warming (West and Sal, 2003). In 2008, the percentage of bleaching was relatively low (10.5%) because of cyclone Nargis and subsequent rainfall and winds which reduced the temperature level by the end of May. There was no coral mortality in 4 years due to elevated SST, but 80% of the bleached recruits died in 2007. Despite this mortality, reefs in the Gulf of Mannar appear surprisingly resilient.

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Annual summer bleaching of a multi-species coral community in backreef pools of American Samoa: a window on the future?

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Abstract. The prevalence of bleaching was recorded in staghorn thickets of two back reef pools on a biweekly basis during the October to June bleaching season, and monthly basis for the rest of the year at Tutuila, American Samoa. Recording began in late 2003 and has continued since, and used a rapid visual estimation procedure. Three species of staghorns (*Acropora spp.*) in both pools bleached every Austral summer for five years. Reports of reef slope bleaching events in 2002 and 2003 imply that they bleached in those years as well. Thus, these corals have bleached for at least five summers and likely seven summers or more in a row. Other, less common species of *Acropora* and *Millepora* were observed to bleach as well. The bleaching displayed patterns consistent with the view that both temperature and irradiance are involved in producing the bleaching. The term "photothermal bleaching" was applied to this type of bleaching. This appears to be the first annual multi-species summer bleaching event reported.

Key Words: bleaching, annual, coral, staghorn, American Samoa

Introduction

Mass coral bleaching events have been reported on many coral reefs around the world (Brown and Ogden 1993; Glynn 1996; Brown 1997, Hoegh-Guldber, 1999, 2000; Wilkinson and Souter 2008). Although most corals recover in some events, in other events, many corals die. The intensity of the 1997-98 mass bleaching event was such that about 16% of the world's corals were estimated to have died in that event alone (Wilkinson et al. 1999). Much evidence links mass bleaching events with increased temperatures, and bleaching is predicted well by sea surface temperatures (SST; Hoegh-Guldberg 1999; McWilliams et al. 2005; Liu et al. 2003). The frequency and severity of events has been increasing (Glynn 1993; Hoegh-Guldberg 1999; Bellwood et al. 2004; McWilliams et al. 2005; Marshall and Schuttenberg, 2006). Global warming has been implicated in the increasing frequency and severity of mass bleaching and mortality events, and events have been predicted to continue to increase in frequency and severity, becoming annual summer events sometime in the future (Williams and Bunkley-Williams 1990; Ware 1997) with most predicting that to occur by about 2020 to 2050 (Hoegh-Guldberg 1999; Done et al. 2003). More severe events such as that in 1998 could devastate reefs by that time even though they would not occur every year, because reefs would require several years to recover from each event (Sheppard 2003).

Coral bleaching events on reef slopes was reported in American Samoa in 1994 (Goreau and Hayes, 1994), 2002 and 2003 (Green 2002; Craig et al. 2005), and caused some coral mortality on reef slopes. SST's for American Samoa show an increasing trend over the last 25 years (US EPA 2007). The observation of bleached staghorn corals in back reef pools of Tutuila, American Samoa in late 2003 prompted the senior author to begin monitoring bleaching in those back reef pools (Craig et al. 2005; Whaylen and Fenner 2006; Fenner et al. 2008; Fenner and Carroll in prep). *Acropora* and *Millepora* are reported to be among the most sensitive genera to bleaching (Marshall and Baird 2000; Loya et al. 2001)

Materials and Methods

Tutuila, American Samoa has nearly continuous fringing reefs on the south shore, with shallow reef flats and steep reef slopes (Craig 2005; Craig et al. 2005; Birkeland et al. 2008; Brainard et al. 2008; Fenner et al, 2008). At a few locations, sections of the reef flat have been dredged to provide fill for the airport runway or village land. These pools reach about 7-10 m depth at their maximum. These back reef pools are now co-dominated by *Porites*

cylindrica and the staghorn *Acropora muricata* (=*formosa*), with smaller amounts of the staghorns *A. pulchra* and *A. nobilis* (referred to as *A. intersepta* in some works). A few other species are scattered in these pools, including at least one stand of *Millepora dichotoma*, and individuals of a few other species of *Acropora*.

Staghorn thickets provide a particularly challenging subject for recording bleaching. Usually, the limits of colonies and clones are not clear. Further, bleaching commonly varies in intensity along the length of branches. In addition, what appear to be clones, and thickets, and different areas of the pools often bleach to different degrees. In most field studies of bleaching, bleaching is recorded by visual estimation. In this study, a rapid visual estimation method was used, in part because limited time was available for this study. A one hour swim over a fixed course was used, passing over two areas with different bleaching rates (Fig. 1), in each of two pools. A running estimate of bleaching was made during the swim, separately for each of the two different areas. At the airport pool, the area nearer to the runway typically was less bleached than the area farther from the runway. In the Alofau pool, the deeper eastern part of the pool always was more intensely bleached than the shallower western part. An area of staghorn was deemed bleached if at least part of branches were light or white on their upper surface only. The intensity of bleaching was not recorded. Bleaching was monitored biweekly when colonies were bleached, and approximately monthly when they were not bleached. Bleaching was also recorded on the reef flat and reef slope at Alofau beginning in January, 2007.

The satellite sea surface temperature (SST) is produced operationally by NOAA Coral Reef Watch in near real-time (http://coralreefwatch.noaa.gov). The SST values are produced globally twice each week and are ~9-km sub-sample averages presented on a 0.5-degree (approximately 50-km) grid (for detail see Skirving et al, 2006). The data used are from the pixel nearest the Airport Lagoon, just to the south, centered at [170.5W, 15.0S]. The warmest month climatology value used by Coral Reef Watch is representative of the expected summertime maximum temperature (and therefore a threshold for bleaching conditions) at this location. While not precisely co-located with the observation site, periods when the SST value exceeds the climatology at the offshore location are representative of when thermal stress is experienced near the coast.

Results

Porites cylindrica was not observed to bleach except at one small area in early 2007 (a patch on the reef flat in Faga'alu Bay). All three of the staghorn species were observed to bleach during the Austral summer at the Airport, Alofau, Nu'uuli, and Faga'itua pools. The Faga'itua pool is a maximum of 2 m deep and may be natural, while the other pools were produced by dredging. Bleaching typically involved lightening of color on the upper surface of horizontal branches, and when intense the entire upper surface was white. Lower surfaces always retained some color, except when a colony died. Polyps on the upper surfaces of a few branches died, while surviving on all of the rest of their surfaces and retaining color on their undersurface. Even during the Austral winter when very few staghorns were bleached, overturned colonies were observed bleached on what was previously their undersurface. Most years there was little if any colony death, but in 2007 in particular, some colonies died all except for branch tips, and a couple of thickets died completely. Thus, this is not just annual summer lightening of color (Fitt et al. 2000). Two of the staghorn species (*A. muricata* and *A. pulchra*) were found only in the back reef pools, but one (*A. nobilis*) was found also on reef slopes. *A. nobilis* was never observed to bleach on reef slopes at the same time it was intensely bleached in the back reef pools. On the reef slope at Alofau, *A. nobilis* on the reef slope at the same depth (about 4-5 m) as in the back reef pool of Alofau, was not bleached in 2007 when the colonies in the pool bleached sufficiently severely that only a few branch tips survived. *M. dichotoma* and some of the *Acropora* species other than the three staghorns were observed to bleach at the same time as the staghorns. A second, much less common bleaching pattern was for several centimeters of branch tips to bleach completely on all surfaces.

Water in the pools was typically warmer than the ocean, and at times could be felt to be warmer near the surface than deeper (the stratification likely due to low-wind conditions). Logger temperature records were obtained from 0.5 and 6 m at the airport pool, which reached maximum temperatures of about 32 °C for short periods midday. In Fagaitua, the logger at 2 m depth recorded a maximum of 34.9 °C for one half hour. That is a temperature similar to the maximum recorded in the back reef pools at Ofu Island in American Samoa, where a high diversity of corals lives (Craig et al. 2001). Staghorns there were moderately bleached at that time.

Staghorns in the Alofau pool bleached every summer (Fig. 1). Data for the deeper eastern area and shallower western area are plotted separately. As can be seen, the deeper area bleached more intensely and for a longer period than the shallow area. Bleaching began each year in October, and ended around the end of May or early June. For the deep pool, the onset

and end of bleaching was rapid, and bleaching was near 100% for most of the Austral summer. Staghorns in the deep pool spent most of the year bleached with only a short respite between years; despite this, corals manage to survive. Bleaching was much less intense on the reef flat, and near zero on the slope.

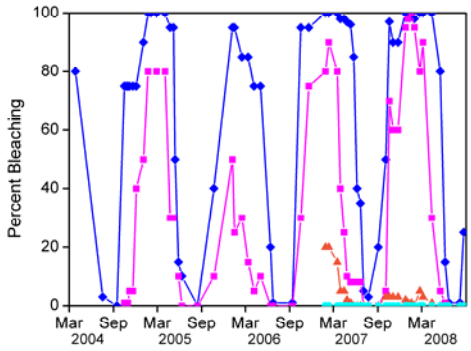

Figure 1 Staghorn bleaching observed at four sites at Alofau – left pool (blue diamond), right shallow (pink square), reef flat (orange triangle), reef slope (cyan circle).

Staghorns in the airport pool bleached each summer as well, and the staghorns spent about as much time bleached as unbleached (Fig. 2). In 2006 and 2008, a period of several weeks of cloudy, rainy, cool weather was followed by a sharp drop in bleaching, and the resumption of normal weather was followed by a resumption of bleaching. Changes in the percentage of corals bleached corresponded closely with nearby ocean SSTs (Fig. 2), appearing to show a rapid, proportional response to warming.

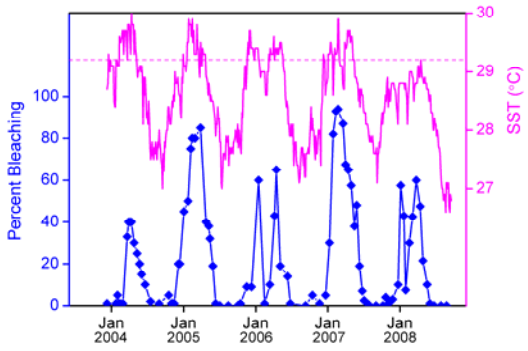

Figure 2 Staghorn bleaching at airport lagoon, Tutuila, and satellite SST. The dashed pink line indicates the temperature of the climatologically warmest month.

Maximum bleaching at the two locations during the five-year period was most often in February with four maximums observed in that month, two in January, two in March, and one in April.

Discussion

Normally, healthy corals show a seasonal variation in the density of their zooxanthellae, with fewer zooxanthellae during the summer and autumn (Fitt et al. 2000). However, healthy corals normally do not loose so many zooxanthellae that they turn white and/or die, so the present observations are beyond the normal range of variation. The pools reached temperatures of about 32 ^{o}C while Fig 2 shows peak temperatures of about 30 ^{o}C for nearby ocean SST's. The fact that *A. nobilis* colonies bleached and partly died in the Alofau pool while the same species at the same depth on the slope about 100 m away did not bleach, indicates that water temperature played an important role in this bleaching, though water motion may well have been a factor as well. The fact that the upper surfaces of staghorns bleached and even died while lower surfaces retained color and survived, is similar to previous reports that upper surfaces of corals bleach more than lower surfaces (Hoegh-Guldberg 1999) and indicates that solar irradiation played an important role in the bleaching as well. This is consistent with the bleaching of the undersurfaces of overturned colonies when other colonies were not bleached, and in addition suggests that upper branch surfaces may have greater resistance to bleaching from irradiation than lower branch surfaces. This pattern is consistent with evidence that bleaching involves the effects of high temperature and irradiation on the photosynthetic system in the zooxanthellae (Coles and Jokiel 1978; Gleason and Wellington 1993; LeTissier and Brown 1996; Hoegh-Guldberg 1999). It is also consistent with reports that previous exposure of coral to bleaching-inducing irradiation can increase resistance to subsequent thermal bleaching (Brown et al. 2000, 2002). The dual roles of irradiation and temperature in bleaching can be captured in the phrase "photothermal bleaching." This also distinguishes this type of bleaching from bleaching produced by other factors, such as darkness, fresh water, or disease. The coral bleaching responded rapidly and proportionately to temperature changes. Maximum bleaching occurred in February, two months after the austral summer solstice, compared to three months after the northern summer solstice in the Caribbean (McWilliams et al. 2005). Thus, bleaching follows peak irradiance and corresponds better to peak SST's in both data sets, indicating that the influence of light is regulated by temperature. Further, there is no sign of the corals developing increasing resistance to

bleaching, even though some in Alofau spend most of the year bleached. However, the low levels of mortality suggest that the corals in these pools may be better equipped to tolerate the effects of bleaching.

Annual summer or autumnal bleaching has been reported in single coral species for *Oculina patagonica* in the eastern Mediterranean (Ainsworth et al. 2007) and *Meandrina meandrites* in Florida (Tichenor 2004). Seven species of intertidal corals have been reported to bleach in Thailand, at least one of which (*Goniastrea aspera*) was documented to bleach annually from solar irradiation (Brown et al. 1994). However, the bleaching of a multi-species coral community in the back reef pools of American Samoa appears to be the first subtidal multi-species bleaching event that appears regularly every summer due to temperature and irradiation. Although this project began at the end of 2003, the fact that bleaching events were reported in 2002 and 2003 indicates that staghorns in the pools very likely have bleached for at least seven years in a row. Such annual bleaching events have been predicted to appear in the future due to global warming (Williams and Bunkley-Williams 1990; Ware 1997; Hoegh-Guldberg, 1999, 2000; Done et al. 2003; Sheppard 2003). These events in the back reef pools of Tutuila may provide insights into the future for coral reefs, and may provide a predictable opportunity to study annual bleaching events and test possible mitigation strategies long before annual summer bleaching becomes common on coral reefs around the world.

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The effect of high and extreme temperature increase on the coral *Porites lutea* and five different algae in northern Vietnam

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Abstract. Global warming and increasing sea surface water temperatures have shown to induce bleaching events and generate phase shifts on coral reefs. Here we investigated the physiological responses (gross primary production, respiration and GP/R ratio) to high temperature (30ºC, i.e. +5ºC above control) and extreme temperatures (34ºC, i.e. +9ºC above control) during 24 hours on one coral species (*Porites lutea*) and five algae species. The result from the organisms' GP/ R ratio showed that only *Sargassum sp.* (*Phaeophyta*) was significantly negatively affected by elevated temperature (34ºC). However, the results showed a positive correlation between the GP/ R ratio and temperature for *Gracilaria asiatica* (*Rhodophyta*). None of the other organisms showed any significant changes in GP/R ratio as a result of treatment. *Asparagopsis taxiformis* (*Rhodophyta*) showed an increase in both gross primary production and respiration. *P. lutea* showed a decrease in both gross primary production and respiration. The physiological responses to increased temperatures differed among the coral reef organisms', even within the same phylum. As a result, this may indicate that the consequences of the imminent global warming may be severe, since it may not only have a negative impact on coral reef organisms directly, but also indirectly, by altering competitive ability between species.

Key words: Increased sea water temperature, physiological responses, algae, *Porites lutea*.

Introduction

Global warming and increasing sea water temperatures have shown to induce bleaching events (Hoegh-Guldberg 1999) and coral mortality (Wilkingson 2004), which furthermore may generate phase shifts on coral reefs (Hughes et al. 2003). In combination with other anthropogenic factors, such as over-fishing (Jackson et al. 2001; Berkes et al. 2006), sedimentation (McCulloch et al. 2003) and pollution (reviewed by Fabricius 2005), this has caused world-wide coral reef degradation (Wilkinson 2000; Jackson et al. 2001).

Coral bleaching causes coral mortality and thereby opens up substratum, which leads to a "window of opportunity" for opportunistic algae to colonize (McClanahan et al. 2001). If herbivores are present they can prevent the algae to overgrow the corals (Hughes et al. 2007). However, if macroalgae successfully establish, a phase shift from coral dominance to macroalgae-dominated state may occur (Done 1992).

On many reefs there have already been transitions from coral dominance to macroalgae dominance due to overfishing of herbivores (Gardner et al. 2003; Hughes et al. 2003; Bellwood et al. 2004). These reefs may therefore lose biodiversity and essential ecosystem services (Nyström et al. 2000) and thus be more vulnerable to climate change (Hughes et al. 2007).

The aim of this study was to investigate the physiological effects of high (30ºC) and extreme temperature (34ºC) increases on one coral species (*Porites lutea*) and five different algae from three different phyla *Ulva compressa, Ulva torta* (*Chlorophyta*), *Gracilaria asiatica, Asparagopsis taxiformis* (*Rhodophyta*) and *Sargassum sp.* (*Phaeophyta*).

Material and methods

P. lutea,, *A. taxiformis* and *Sargassum sp*. were collected at Long Chau Island, Halong Bay (N 20˚ 37' 27", E 107˚ 09' 41") in northern Vietnam. *U. compressa*, *G. asiatica* and *U. torta* were collected in aquaculture ponds close to the lab. Both coral and algae organisms had 72 hours to acclimatize to the lab environment before the experiment started. The experiment was carried out during March 2007 in an outdoor lab at Tram Bien Research Station in Do Son, northern Vietnam.

For the experiment 3 large tanks (1 m^3 each) were used in order to minimise temperature fluctuations (Nyström et al. 2001; Nordemar et al. 2003). In each tank four aquaria (20 l each) were placed. In each aquarium 14 coral pieces or algae tissue were placed

using a randomized block design. During measurements the corals and algae were individually placed in separated transparent jars with air-tight lids, containing 1.5 litre of water each, with the same temperature as the exposure treatments. The three tanks represented: a) control (25ºC), b) high increased temperature (30ºC, i.e. +5ºC above control) and c) extreme increased temperature (34ºC, i.e. +9ºC above control).

In the two exposure tanks, the temperature was increased by applying external heating regulators (ZEBO 300W or FLUVAL Tronic 200W). Aeration pumps mixed the sea water in both the aquaria and the tanks, thus maintaining high oxygen concentrations and distributing water temperature evenly. The experiment was conducted during natural moderate light conditions and the irradiance was 700 (±308) $\mu E/m^2/s$ during the measurements.

The organisms were exposed for 24 hours. Thereafter changes in dissolved oxygen concentration were measured in light (net production) and in darkness (respiration) according to Moberg et al. (1997) using an oxymeter (WTW Oxi 330). To measure the net production, the oxygen level was noted initially and the containers were placed outdoor in full daylight. The oxygen levels were noted again after 30 minutes. To measure the respiration, the organisms were placed in darkness for 30 minutes to adapt to darkness and stop primary production (Moberg et al. 1997), before the initial oxygen value was measured. The oxygen levels were measured once more after 2 hours in darkness.

Before the statistical analyses, the volume of the whole coral was measured by submerging the coral into a beaker filled with a known volume of water, to compensate for volume dependent differences in dissolved oxygen concentration. Thereafter the coral's surface was measured by using the aluminium foil method (March 1970).

Gross primary production rate and respiration rate data were calculated per hour and per cm^2 coral surface area (Moberg et al. 1997) and expressed as O_2 (mg)/h*cm^2. Furthermore, the dry weight of the algae was noted and then the gross primary production and respiration rates were expressed as O_2 (mg)/h*dry weight (g).

For both corals and algae the respiration rate was added to the net production value in order to achieve gross primary production rate (here after referred as gross production). Gross primary production/ respiration (GP/R) ratios were also calculated.

A one-way ANOVA was performed to analyse the effects of treatments for each species. Where the one-way ANOVA showed differences, Tukey HSD was used as a post hoc test ($p<0.05$). To investigate correlations in the data, Pearson correlation test was used. All data were tested for homogeneity of variances using Levene's test ($p<0.05$) before the ANOVA was carried out. For *U. compressa* and *U. torta* only the data from the control and 30ºC treatment group was included in the statistical analysis, since the values from the 34ºC treatment group were considered inaccurate. A t-test was use as a statistical test. For the statistical analyses the software SPSS 13.0 edition for Mac OS X was used.

Results

GP/R ratio

The result from the organisms' GP/ R ratio showed that *Sargassum sp.* was negatively affected by elevated temperature ($p<0.001$, $r=-0.893$ Pearson correlation) (Fig. 1). The 30ºC treatment ($p<0.05$, Tukey HSD) and the 34ºC treatment ($p<0.001$ Tukey HSD) had a lower GP/R ratio compared to the controls (Fig. 1).

For *G. asiatica* the GP/ R ratio showed a trend with a higher GP/R for the algae in the 34ºC treatment group compared to the controls ($p=0.071$ Tukey HSD) (Fig. 1). There was also a positive correlation between the GP/ R ratio and temperature for *G. asiatica* ($p<0.05$, $r=0.488$ Pearson correlation). None of the other organisms (*P. lutea*, *A. taxiformis, U. compressa and U. torta*) showed any variation in GP/ R ratio as a response to high or extreme temperature increase (Fig. 1, 4).

Gross primary production rates

The results from the gross production rates showed that increased temperature had variable effect on the coral and the algae species.

The gross production rate increased significantly with increased temperature in *A. taxiformis*, for both the 30ºC ($p<0.05$ Tukey HSD) and the 34ºC treatment group ($p<0.001$ Tukey HSD) (Fig. 2).

A different result was found in *Sargassum sp*., where the gross production rates decreased in the 34ºC treatment group compared to the control, however this was not significant compared to the controls ($p=0.053$ Tukey HSD). However, the 34ºC treatment group had a lower gross production rate compared to the 30ºC treatment group ($p<0.01$ Tukey HSD) (Fig. 2).

P. lutea showed a lower gross production rate in the 34ºC exposure compared to the controls, however, this was not significant ($p=0.058$ Tukey HSD).

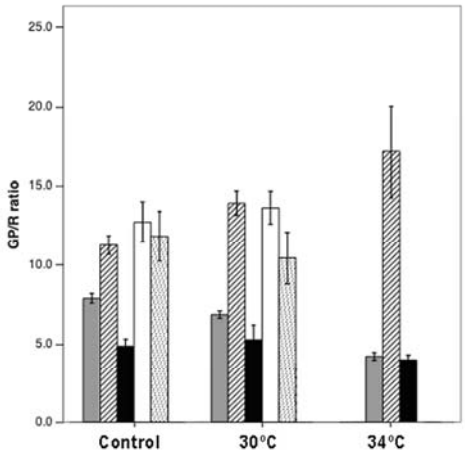

Figure 1: GP/R ratio. *Sargassum sp.* (grey): Control vs. 30ºC *, Control vs. 34ºC ***. *Gracilaria asiatica* (striped): Control vs. 34ºC trend p=0.071. *Asparagopsis taxiformis* (black), *Ulva torta* (white), *Ulva compressa* (dotted): ns. n=7. Error bars: SE.
* p<0.05, *** p<0.001, ns= non significant.

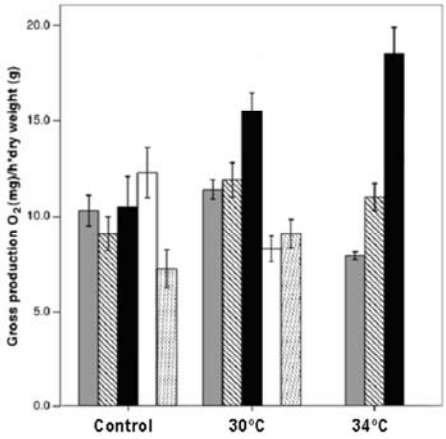

Figure 2: Gross primary production rates. *Asparagopsis taxiformis* (black): Control vs. 30ºC *. Control vs. 34ºC ***. *Sargassum sp*. (grey): Control vs. 34ºC trend p=0.053. 30ºC vs. 34ºC **. *Gracilaria asiatica* (striped), *Ulva torta* (white), *Ulva compressa* (dotted): ns. n=7. Error bars: SE. * p<0.05, ** p<0.01, *** p<0.001, ns= non significant.

Furthermore, there was a significant difference between the higher gross production rate in the 30ºC exposure compared to the 34ºC exposure group (p<0.01 Tukey HSD) (Fig. 4). There were no significant results in gross production rates for *U. torta*, *U. compressa* or *G. asiatica* (Fig. 2).

Respiration rates

Sargassum sp. showed an increase in respiration rate as a reaction to increased temperatures, with a significant difference between the control and the 34ºC exposure group (p<0.01) (Fig. 3).

The respiration rates increased in *A. taxiformis* with temperature and showed a significant difference between the control and the higher respiration value in the 34ºC treatment group (p<0.001), there was also a trend for an increase in respiration in the 30ºC treatment compared to the control (p=0.051) (Fig. 3).

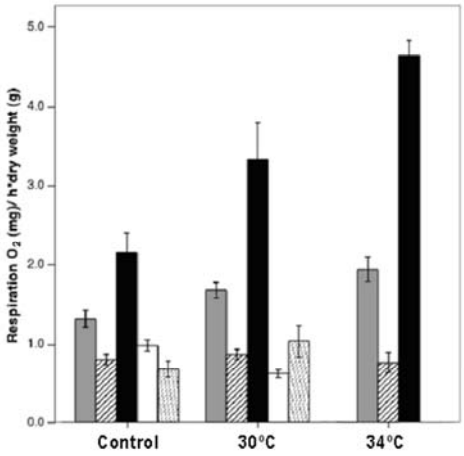

Figure 3: Respiration rates. *Sargassum sp.* (grey): Control vs. 34ºC **. *Asparagopsis taxiformis* (black): Control vs. 30ºC trend p=0.051. Control vs. 34ºC ***. 30ºC vs. 34ºC *. *Ulva torta* (white): Control vs. 30ºC **. *Gracilaria asiatica* (striped), *Ulva compressa* (dotted): ns. n=7. Error bars: SE. * p<0.05, ** p<0.01, *** p<0.001, ns= non significant.

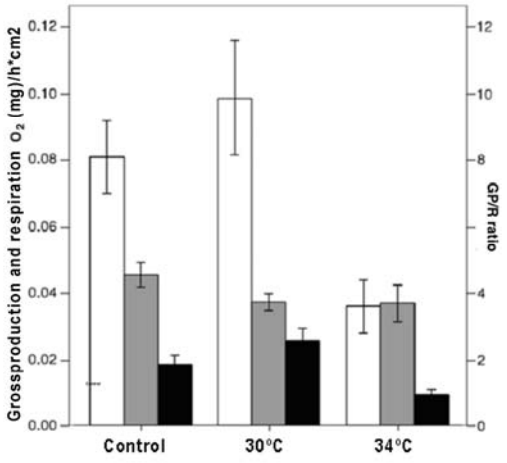

Figure 4: GP/R ratio, gross primary production and respiration rates for *Porites lutea*. Gross primary production (white): Control vs. 34ºC trend p=0.058. 30ºC vs. 34ºC **. Respiration (black): Control vs. 34ºC trend p=0.082. 30ºC vs. 34ºC **. GP/R ratio (grey): ns. n=7. Error bars: SE. ** p<0.01, ns= non significant.

Furthermore, the 34ºC treatment had a higher respiration compared to the 30ºC treatment (p<0.05) (Fig. 3). *U. torta* showed a decrease in respiration rates between the control and the 30ºC treatment group (p<0.01) (Fig. 3).

The respiration rate for *P. lutea* in the 34ºC treatment group was lower than both the control ($p=0.082$) and the 30ºC treatment group ($p<0.01$) (Fig. 4).

No significant differences could be found in *G. asiatica* or *U. compressa* (Fig. 3).

Discussion

Several authors have reported that increased temperature may have a negative effect on corals (Coles and Jokiel 1977; Fitt and Warner 1995; Hoegh-Guldberg and Smith 1989; Nyström et al. 2001). However, studies have shown a positive correlation between increased temperature and GP/R ratio in algae (Elwing and Tedengren 2000) and increased algae biomass (Tsai et al. 2005).

The results from the study showed dissimilar responses in GP/R ratio to elevated temperature for the algae species. *Sargassum sp.* was negatively affected by increased temperature, which was reflected in a decrease in GP/R ratio. In the field, Ateweberhan et al. (2005) showed that the highest growth rate for *Sargassum sp.* occurred at 28-30ºC, while the growth rate was below zero when the temperature reached 33-36ºC. Furthermore, *Sargassum sp.* has shown to be a late succession species (Hughes et al. 2007), which establish after green filamentous algae (Kokita and Nakazono, 2001), such as *Enteromorpha* (McClanahan 1997). Our results showed that neither *U. torta* nor *U. compressa* was negatively affected by a high increase in temperature. This suggests that increased sea water temperature may have a limited effect on early succession algae species, which may or may not be followed by a succession of brown frondous algae.

In contrast to *Sargassum sp.*, *G. asiatica* was positively affected with an increase in GP/R ratio, in response to elevated temperature. A decrease in gross production rate together with an increase in respiration rate explained the changes in GP/R ratio for *Sargassum sp.*, while the result for *G. asiatica* was explained by a stimulated gross production rate whereas no change in respiration was noted. A positive relationship between temperature and growth rate for *Gracilaria coronopifolia* has been found by other authors (Tsai et al. 2005). Furthermore the results showed that *A. taxiformis* increased both gross production and respiration rates due to the temperature treatment, however the GP/R ratio was not affected. This in turn might indicate higher metabolic requirements and energetic costs due to thermal stress (Bayne et al. 1985). On disturbed coral reefs, *Asaparagopsis taxiformis* may completely dominate the reef and form large canopy (Diaz-Pulido and McCook 2002), which, especially in the absence of herbivores, may prevent corals to re-establish after disturbances (Hughes et al 2007).

In this study *P. lutea* did not show any changes in GP/R ratio due to heat treatment. Nevertheless, both gross production and respiration rates were affected, indicating that increased temperature had an effect on the coral's metabolism, at least to some extent.

A possible explanation to these results may be that the corals in the region may be adapted to large variation in seasonal temperature, as has been noted in other regions (Kemp et al. 2006), since the water temperature may fluctuate between 16ºC and 31ºC in the Halong Bay area during the year (Nguyen 2004; Latypov 2005). Upper thermal limits have been shown to vary between the same coral species in different regions (Hughes et al. 2003), suggesting that local adaptation may occur (Cook et al. 1990).

Furthermore, *P. lutea* has been shown to be tolerant to elevated temperatures and has been known to prevail during bleaching events (Loya et al. 2001; Baker et al. 2004; McClanahan et al. 2007). Nevertheless, our results suggest that since increased temperature in fact benefited several of the algae species, but had a limited effect on *P. lutea*, this may alter the competition between the species.

The results from this experiment demonstrate that coral reef organisms' physiological responses to increased temperatures vary between species, even within the same phylum. This in turn suggests that the consequences of global warming may be severe, since it not only will affect coral reef organisms directly, by reduced metabolism/growth rate and increasing mortality, but also indirectly by altering their relative competitive ability, which in turn may lead to decreased biodiversity and an increase in coral reef vulnerability to further anthropogenic or natural disturbances.

In addition, during periods of elevated temperatures, this could have further implications. If several species of algae may in fact be positively affected by elevated temperatures this may lead to a more rapid overgrowth of algae after e.g. bleaching events, and thereby contribute to a phase shift, especially in areas where herbivore fish and invertebrates are more or less absent, due to over-exploiting of marine resources.

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Reef development and resilience to acute (El Niño warming) and chronic (high-CO_2) disturbances in the eastern tropical Pacific: a real-world climate change model

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Abstract It has been recently recognized that eastern tropical Pacific (ETP) coral reefs exist under naturally-occurring high-CO_2, low carbonate saturation (Ω) conditions that encompass the range of expected changes for the entire tropical surface ocean with a doubling and tripling of atmospheric CO_2. Holocene reef development positively and linearly tracks Ω in the ETP; illustrating the real-world importance of this variable on reef building. Galápagos reef communities have been subject to the most extreme thermal anomalies associated with the El Niño-Southern Oscillation (ENSO) and also experience the lowest Ω levels in the ETP. Reef resilience has been so poor in Galápagos that reef structures were completely bioeroded in < 10 years after the 1982-83 ENSO. The closure of the Panamanian isthmus 3-3.5 million years ago created the present-day oceanographic conditions responsible for ENSO-related warming events and the upwelling of high-CO_2 waters throughout the ETP. These combined acute (ENSO) and chronic (high-CO_2) disturbances may help explain why coral reefs are scant and many genera of corals went extinct in the ETP during the late Cenozoic; thus providing a real-world example of the combined thermal and chemical ramifications of climate change on coral reef structure, function and resilience to disturbance over geologic time.

Keywords Ocean acidification, Eastern tropical Pacific, Climate change, Reef development

Introduction

Coral reefs flourish in the tropics where temperatures are warm year-round (> 18°C), illumination is high/turbidity is low, and seawater is supersaturated with respect to calcium carbonate ($CaCO_3$) (Kleypas et al. 1999a). Considerable environmental variability has been recognized as detrimental to coral reef development since Charles Darwin's (1842) original treatise on the subject. The relative importance of the specific environmental variables eliciting this distributional hypothesis is less obvious. Elevated nutrients have been hypothesized to be particularly deleterious to coral reef development based upon the assumptions that nutrients stimulate water-column productivity and turbidity, thus limiting the light required by the photosynthetic endosymbionts of reef corals (Hallock 1988) (Fig. 1). Increased levels of nutrients and productivity are thought to favor the proliferation of fleshy benthic macroalgae (autotrophs) and heterotrophic communities (e.g. filter-feeding bioeroding organisms), respectively, at the expense of "mixotrophic" community assemblages like that of modern-day Scleractinian coral reefs (Hallock and Schlager 1986) (Fig. 1). Also, Hallock and Schlager (1986) suggested that high concentrations of phosphate inhibit coral calcification given its potential to act as a poison to crystal formation (Simkiss 1964).

However, nearly all the evidence supporting this hypothesis is qualitative (e.g. Hallock and Schlager 1986; Hallock 1988) or high nutrient exposure experiments only elicit significant results on coral calcification at concentrations that would rarely, if ever occur in nature (Kinsey and Davies 1979; Ferrier-Pagès et al. 2000). Coral reefs do, in fact, persist under wide ranges of nutrients and turbidity (Smith and Jokiel 1978; Perry et al. 2008) and even reef areas thought to be oligotrophic experience variable and sometimes high levels of dissolved nutrients due to natural physical forcing mechanisms (Leichter et al. 2003). Several recent review papers have pointed out the tenuous links between nutrients, algal competition and coral reef decline (McCook et al. 2001; Szmant 2002). As Tomascik and Sander (1985) suggested, it is likely that nutrients themselves do not elicit the most negative effects *per* se, but rather the increase in water-column productivity, turbidity and accompanying decline in light levels

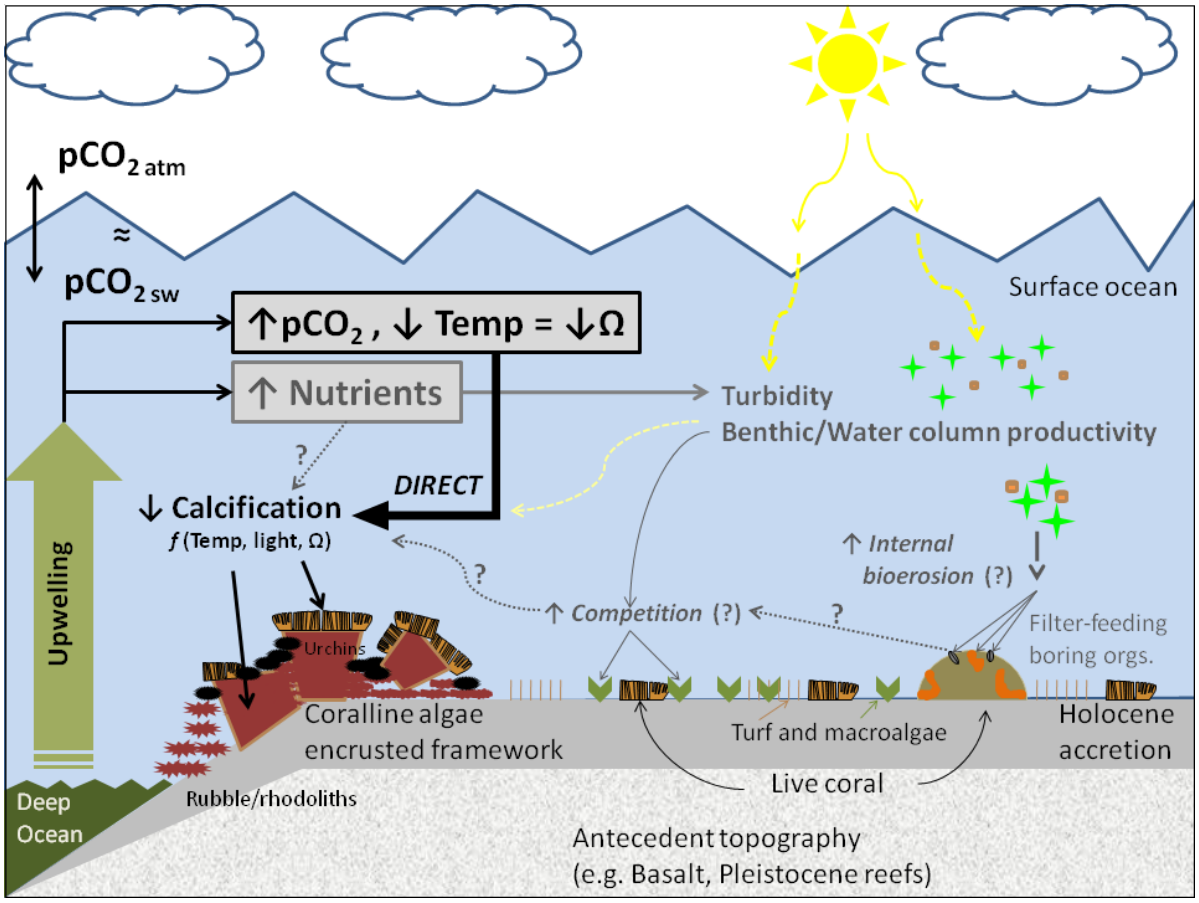

Fig. 1. Simplified model illustrating direct effects of high-pCO_2, low-Ω seawater on reef-building in an upwelling environment compared to primarily indirect effects of elevated nutrients (Smith and Buddemeier 1992).

that are associated with nutrient inputs (natural and anthropogenic) are most deleterious for reef corals.

Upwelling and reef development in the eastern Pacific

Upwelling areas experience highly heterogeneous environmental conditions that experience not only wide ranges in temperature, nutrients and turbidity, but recently it has been recognized that upwelled waters have a high partial pressure of carbon dioxide (high-pCO_2) (Takahashi et al. 1997). This high-pCO_2 effectively depresses the saturation state of $CaCO_3$ ($\Omega = [Ca^{2+}][CO_3^{2-}]/K'_{sp}$, where K'_{sp} is the solubility product of a carbonate mineral such as aragonite or calcite), which is a function of both CO_2 and temperature. Indeed, surface waters in many parts of the eastern tropical Pacific (ETP) have lower pH, lower Ω, and higher pCO_2 values relative to the rest of the tropics (Manzello et al. 2008). Given that depressed Ω and temperature elicit a direct negative effect upon coral calcification and reef building (Langdon et al. 2000), it is hypothesized that these two variables may be just as, if not more responsible than nutrients for the paucity of reef development in upwelling zones.

The intensity of upwelling varies regionally and strongly influences reef development across the ETP (Cortés 1997). High-CO_2, low-Ω influenced coral reefs of the ETP are poorly cemented accretions of $CaCO_3$ that are subject to rapid bioerosion (Manzello et al. 2008; Fig. 2a). The thickness of these reefs reflects Ω in a positive, linear fashion (Fig. 3), illustrating the net result of coral calcification ($CaCO_3$ production) minus its loss (erosion) over geologic time. Fig. 3 illustrates the extent of reef framework accumulation across a naturally-occurring gradient of seawater chemistry, but does not imply a direct cause and effect relationship as there are several additional physical variables that significantly influence reef growth and development such as temperature, light, shelf-area, and antecedent topography (Macintyre et al. 1992). Despite these caveats, it can be reasonably deduced that the effects of high-CO_2 most clearly manifest over geologic time-scales in the processes of reef framework development, preservation potential, and persistence (reef cementation: Manzello et al.

2008; framework accumulation: Fig. 3), rather than in the readily observable biological phenomena on the reef surface.

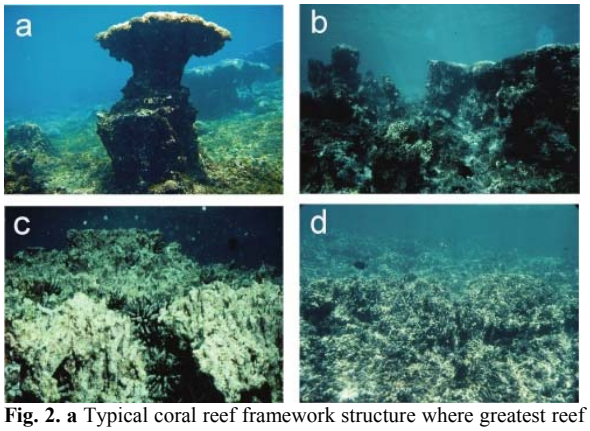

Fig. 2. a Typical coral reef framework structure where greatest reef development occurs in the far eastern Pacific: Secas Islands, Gulf of Chiriquí, Panamá. Vertical relief of framework structure is ≈ 1.5 m. **b** Galápagos coral reef prior to 1982-83 El Niño-Southern Oscillation (ENSO) in 1976. **c** Rapid bioerosion of dead reef framework by echinoids (*Eucidaris galapagensis*) in 1987. **d** Galápagos reef framework completely bioeroded to rubble and sediment by 1992. Images **a** and **b-d** taken by and courtesy of T. B. Smith (Univ of Virgin Is) and P. W. Glynn (RSMAS, Univ of Miami), respectively.

Ocean acidification and global warming analogs in the eastern Pacific

Anthropogenic forced climate change models predict continued and accelerated global warming due to rising concentrations of CO_2, primarily from the unregulated combustion of fossil fuels since the industrial revolution (IPCC 2007). Global mean surface temperature has increased approximately 0.74°C in the past 100 yrs and is expected to increase by no less than 1.5°C further by the year 2100 (IPCC 2007). Proposed increases in temperature are of concern because reef-building corals live at or near their upper thermal limits as positive temperature anomalies of as little as 1°C sustained for one month during the warmest part of the year have been shown to correlate with mass-coral bleaching events (Glynn 1993; Goreau and Hayes 1994; Brown 1997).

An estimated one-third of all the CO_2 released into the atmosphere since the industrial revolution has been absorbed by the oceans (Sabine et al. 2004). This ongoing and accelerating uptake of atmospheric CO_2 is causing a drop in seawater pH at the global scale (Orr et al. 2005), resulting in an acidification of the surface ocean (Caldeira and Wickett 2003). Ocean acidification results in a decrease in seawater $[CO_3^{2-}]$ and, consequently, a decrease in the saturation state (Ω) of carbonate minerals (Kleypas et al. 1999b). Acidification is expected to reduce coral reef calcification and increase reef dissolution, and the relative rates of change will likely be a function of pCO_2 in surface seawater, which is near equilibrium with pCO_2 in the atmosphere (Smith and Buddemeier 1992; Kleypas et al. 1999b; Langdon et al. 2000; Yates and Halley 2006). Carbonate budget studies have shown that healthy coral reefs exhibit low net accretion due to high rates of physical, chemical and biological erosion (reviewed by Glynn 1997a). Consequently, any disturbance that causes either decreased accretion or increased erosion may tip the tenuous balance from reef framework growth to loss.

The carbonate chemistry of seawater influencing reef sites in the Galápagos and Gulf of Panamá is similar to what is expected for the average tropical surface ocean with a doubling and tripling of atmospheric CO_2, respectively (Fig. 3). Thus, the response of these reef communities to the El Niño-Southern Oscillation (ENSO) provides a real-world example of what may be the future for coral reefs globally with accelerating climate change.

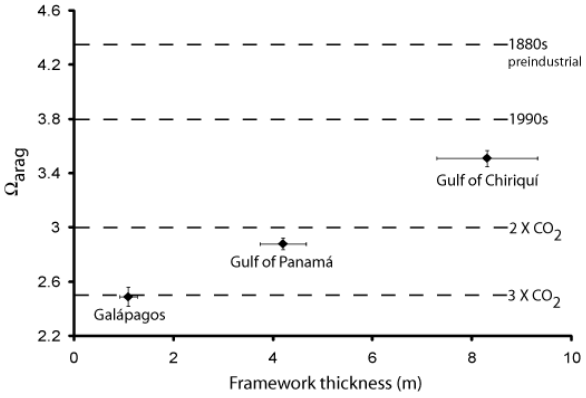

Fig. 3. Mean maximum reef framework thickness (m, ±SEM) from eastern tropical Pacific relative to the saturation state of aragonite, Ω_{arag}. Aragonite is the type of $CaCO_3$ deposited by Scleractinian corals. Ω_{arag} values measured in ETP and estimated for average tropical surface ocean with differing levels of atmospheric CO_2 calculated as described in Manzello et al. (2008). Framework thicknesses of all pocilloporid reef sites prior to the 1982-83 ENSO in Galápagos and Panamá originally presented by Glynn and Wellington (1983) and Glynn and Macintyre (1977), respectively.

Galápagos coral reef communities experienced a greater and longer thermal anomaly (3-4°C for several months) associated with the 1982-83 ENSO in comparison to those in Pacific Panamá (1-2°C for two months) (Podesta and Glynn 1997). As a result, coral mortality from prolonged bleaching was greater in Galápagos (97-99%) compared to Panamá (75-85%; Glynn 1990). Following this mass mortality, reef framework structures in Galápagos were rapidly and completely bioeroded to rubble and sand in less than a decade and are now non-existent (Glynn 1994; Reaka-Kudla et al. 1996; Fig. 2b-d). Conversely, reef framework structures have persisted in Panamá despite evidence of net erosion following two severe

ENSO events (Eakin 2001). The rapid destruction and disappearance of Galápagos reefs is the end result of several cascading factors that include the extreme thermal anomaly/resultant mass mortality, less pre-existing amounts of reef framework compared to Panamá, a population explosion of bioeroding echinoids, and poor recruitment potential (Glynn 1994). However, the impact of high-CO_2, low-Ω seawater on carbonate cement precipitation and its apparent inverse relationship to bioerosion rate in the ETP adds a key piece to the puzzle as to why reefs throughout the ETP are poorly developed and ephemeral on geologic time-scales (Manzello et al. 2008).

Durham's dilemma revisited

The closure of the Panamanian isthmus 3 to 3.5 million years ago established the present-day oceanographic conditions (Keigwin 1982) necessary for ENSO-related climate phenomenon (Colgan 1990). These oceanographic conditions also established the chronic upwelling of high-CO_2, sub-thermocline waters to the surface layers throughout the ETP (Keigwin 1982; Takahashi et al. 1997; Manzello et al. 2008). Even where ETP reefs are best developed in the Gulf of Chiriquí off Panamá, the signature of these high-CO_2, low-Ω waters is evident at shallow depths (Table 1). These significant decreases in Ω, temperature, and increases in turbidity and nutrients at shallow depths illustrate why reefs are generally restricted to very shallow depths (< 10 m) throughout the ETP (Dana 1975; Glynn and Maté 1997; Cortés 1997; D'Croz and O'Dea 2007).

Table 1. Carbonate parameters measured *in situ* at surface (n = 82) and 15 m depth (n = 13) at the Uva Reef, Gulf of Chiriquí, Panamá. Values represent means (±SEM) of samples taken during both wet and dry seasons over four consecutive years (2003-2007).

Depth	TCO_2 (μmol kg^{-1})	TA (μmol kg^{-1})	pH (sws)	pCO_2 (μatm)	Ω_{arag}	T (°C)
< 5 m	1795.3 (9.27)	2089.6 (8.45)	8.01 (0.01)	406 (14.1)	3.51 (0.06)	28.9 (0.11)
15 m	1931.0 (18.8)	2187.5 (15.4)	7.96 (0.012)	480 (16.5)	3.05 (0.09)	25.9 (0.77)
†P	***	***	*	**	**	***

†-Probability that two distributions were not significantly different using Mann-Whitney U-tests (P < * = 0.01, ** = 0.001, *** < 0.0001). TCO_2, TA and *in situ* temperature measured and CO_2-system calculated as described in Manzello et al. (2008).

J. W. Durham (1966) posited two questions, later referred to by Glynn (1997b) as 'Durham's dilemma', related to the depauperate state of coral reefs in the far eastern Pacific: (1) Why are coral reefs absent during the late Cenozoic and (2) why did so many genera of corals go extinct in the ETP during the Cenozoic, but continue to persist in other areas? Glynn (1997b) suggested that the ENSO-induced thermal anomalies that can elicit mass-mortalities, severe population reductions, and extirpations of reef corals throughout the ETP were likely a pivotal element towards resolving this dilemma.

Veron (2008) recently hypothesized that perturbations to the global carbon cycle and ocean acidification provide an explanation for past episodes of reef-building extinction (i.e. reef gaps) in the geologic record. The interaction of oceanography, biology, and geology in response to ENSO across the ETP illustrates one mechanism for the cessation of reef-building and destruction of reef structures.

The chronic influence of these previously unappreciated low-pH, low-Ω, and high-pCO_2 waters acting in concert with periodic, acute ENSO-associated thermal anomalies may provide an answer to Durham's dilemma while simultaneously granting a real-world model for the combined thermal and chemical ramifications of climate change on coral reef structure (Manzello et al. 2008), function and resilience to disturbance over geologic time.

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Bleaching as a life history trait in coral-zooxanthellae holobionts – relevance to acclimatization and adaptation

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Abstract. This paper explores the relationship between bleaching patterns and growth strategies of coral-zooxanthella holobionts in relation to tradeoffs in benign and stressful environments. Growth strategies can be differentiated between growth/production maximizing versus stress tolerating/persistent options. The former are dependent on high growth (or reproductive) rate for competitive success and extension over space; their tradeoff is a high opportunity cost of reducing production, i.e. of an adversity response when stressed. The latter are dependent on their ability to persist under adverse conditions to maintain control over space; their tradeoff is low performance under good conditions. Obura's (2009) model for stress resistance in corals predicts that bleaching is induced under stressful conditions to reduce excess production by zooxanthellae and thereby maintain symbiotic balance. This hypothesis is consistent with bleaching responses where corals that invest in rapid growth (/reproduction) tend to bleach and die at lower levels of stress (e.g. *Acropora, Pocillopora*), while corals that invest in stress resistance tend to bleach and survive at higher levels of environmental stress (e.g. *Porites*). These bleaching/life history strategy patterns shed light on the scope for acclimatization and adaptation of corals, independently of the multiplicity of mechanisms that are precursors to the bleaching response.

Keywords: bleaching, symbiosis, scleractinian coral, zooxanthellae, climate change, environmental stress

Introduction

Obura (2009) presents a model for coral bleaching that reconciles the adaptive role of bleaching (Fautin and Buddemeier 2004) with the many competing interpretations of bleaching in the literature (Hoegh-Guldberg 2005) (Fig. 1). The model is based on life history tradeoffs between fast growth and reproduction versus stress resistance and persistence (Grime 1977, Stearns 1989, Greenslade 983) and interpretation of bleaching as a Stress Response Syndrome (SRS) or General Adaptive Mechanisms (Stebbing 1981).

The model is based on the premise that the life history strategies of the space-dominating (e.g. *Acropora*) and opportunistic strategies of small branching (e.g. pocilloporid) corals require high investment in maintaining high photosynthetic activity by zooxanthellae and consequently high metabolic activity of the holobiont to utilize and manage this energy. Conversely, the stress tolerant strategies characterized by large long-lived massive (e.g. *Porites*) and many small slow-growing (e.g. *Siderastrea*) corals require commitment to an adversity response to stress, i.e., the ability to survive at low metabolic levels during stress to

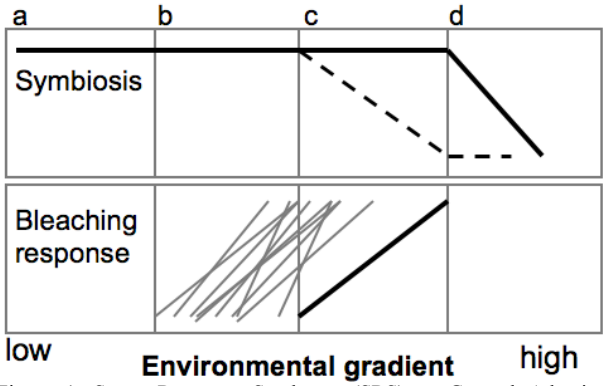

Figure 1. Stress Response Syndrome (SRS) or General Adaptive Mechanism (GAM) model (Stebbing 1981) for coral bleaching (Obura 2009). The intact symbiosis is maintained in balance (panels a-c, top) by homeostatic control mechanisms, but declines to zero (mortality, panel d) when the control mechanisms are exhausted. At low levels of stress a variety of first order stress responses (e.g. photo-chemical quenching, induction of heat shock proteins) sensitive to environmental stimuli (grey lines, panel b, bottom) may be induced, depending on the external stress. Once their tolerance range (counteractive capacity) is exceeded a second order control system sensitive to internal stress, the bleaching response, is activated (panel c, bottom), during which coral colour decreases (dotted line, top panel), but the symbiosis is intact and health (solid line, panel c, top). Once the maximum threshold for bleaching is exceeded, homeostasis is impaired, and the holobiont begins to break down irreversibly (panel d). Adapted from Obura (2009).

Table 1. Life history strategies based on the primary literature on animals and plants, and observational/empirical strategies described for stony corals.

Equilibrium/Competitive	*Opportunistic/Ruderal*	*Persistence/Adversity*	*Source(s)*
Primary models			
Density dependence, low rates of increase, thrive in predictable conditions.	Density independence, high rates of increase, thrive in unpredictable conditions.	Adaptation to predictably unfavourable environments. Slow rates of increase, low abundance, persistence.	MacArthur 1960, Pianka 1970, Greenslade 1983
Highly competitive, large size, fast growth.	Opportunistic, small size, fast growth, ephemeral.	Stress-tolerant, varied growth forms, slow growth, long lifespan.	Grime 1977
Empirical coral models			
N/a	Opportunistic, fast growth rates, high sexual and/or asexual reproduction, monopolize space in short term. High turnover. *Acropora, Pocillopora*.	Adaptations to low abundances, persistence through varied environmental conditions. *Porites*.	Cameron and Endean 1990
Branching, high reproductive output (spawners), low recruitment, high survivorship of juveniles, high fragmentation, high regeneration fast growth rates, long-lived but with senescence. *Acropora*.	Foliaceous/branching, high recruitment, fast growth, high tissue turnover, high partial and full mortality, small adult size, low competitive abilities, low regenerative abilities. Small agariciids, pocilloporids.	Massive, low recruitment, high survivorship, slow growth, low partial mortalit, fragmentation important, long life expectancy, low tissue turnover, large adult size, competitively aggressive, resistant to sediment , high regeneration. *Montastrea annularis, Porites*	Bak and Engel 1979, Jackson and Hughes 1985, Kojis and Quinn 1994

endure it until conditions improve (Greenslade 1983, Hoffman and Parsons 1991). These expectations are consistent with predictions of life history theory (Stearns 1992), which distinguishes between strategies that maximize or invest in growth and/or reproduction (production) vs. those that maximize survival through stress resistance (persistence). These strategies impose opposite constraints on many organismal processes, and require investments from a limited resource base (Stearns 1989). In the terms of the two main strategy concepts explored in the plant and animal literature – the r-K theory of Macarthur (1960), and the CSR theory of Grime (1977), these can be expressed as opposite responses with respect to energetic investment of the life history strategy – high-energy investment in rapid growth and dominance of space – K or C strategies, and/or high levels of reproduction – r or R strategies, and low-energy investment in longevity – K, or stress resistance – S strategies.

The literature on coral life history strategies has focused on life history traits such as recruitment rate, growth rate, colony size, colony morphology, longevity and reproductive strategies, with authors variously identifying 2 or 3 basic strategies (Table 1). Obura (2001) interpreted these with respect to bleaching patterns to distinguish the high-energy and low-energy options above, but an explanatory framework explaining why this should be so has been lacking. Obura (2009) proposes bleaching as a last-resort mechanism that the coral-zooxanthellae holobiont uses to manage energy relations consistent with the above strategies, and this paper explores this framework for understanding variability in bleaching patterns across taxonomic, spatial and temporal domains.

Methods

The hypothesized role of bleaching is to slow down zooxanthellar photosynthesis as an adversity response under stress, acting as a Stress Response Syndrome or General Adaptive Mechanism (Stebbing 1981). Explicit gene-environment interactions and selective pressures on bleaching as an SRS/GAM can be deduced and tested, against predictions for growth/reproduction or stress resistance based on life history theory (Stearns 1992). In scleractinian corals, growth and reproductive output are determined by zooxanthellae production and translocation rates, as these are energy intensive processes. Thus a direct relation between bleaching and reduced growth/reproduction can be inferred and used to interpret tradeoffs in life history strategies between high production (growth, reproduction) vs. stress resistance (persistence, adversity).

The paper will analyze hypothetical changes from a baseline state of a coral-zooxanthellae holobiont in the parameters of the bleaching response – the induction and maximum thresholds and counteractive capacity. Two basic responses of the bleaching parameters will be explored:

A. Linear shifts up and down the stress gradient of induction and maximum thresholds, maintaining constant counteractive capacity (slope and range from induction to maximum), and
B. Non-linear shifts up and down the stress gradient of induction and maximum thresholds, resulting in varying counteractive capacity, of the bleaching response.

These changes represent selective pressures imposed by environmental conditions on different life history strategies, and the hypothesized impacts on bleaching and holobiont survival will be analyzed for consistency with field observations and life history theory.

Results

A linear shift up or down the environmental axis, of both induction and maximum bleaching thresholds results in no change in the slope of the response, and no change in its counteractive capacity (Fig. 2a, Table 2). The resulting new bleaching responses are therefore:

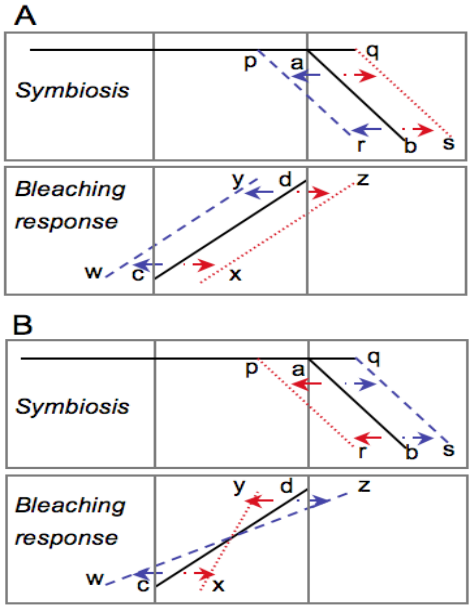

Figure 2. Bleaching response and symbiosis state – linear (A) and non-linear (B) shifts in response thresholds. Solid lines represent intial state, dotted lines are adaptive responses, see text and Table 2 for details.

Table 2. Summary of bleaching response and symbiosis states from Fig. 2, and see text for details.

Bleaching response	Symbiosis state	Growth rate	Stress resistance	Response shift
w-y	p-r	slow	low	Linear
x-z	q-s	fast	high	Linear
x-y	p-r (<r?)	fast	low	Narrowed?
w-z	q-s (>s?)	slow	high	Broadened?

- down-shift on the environmental axis (bleaching response c-d to w-y; symbiosis response a-b to p-r). The effect of this is less growth (earlier shut-down of maximum growth at w-y vs. c-d) and lower resistance to environmental stress (bleaching and mortality at lower levels of external stress p-r vs. a-b).
- up-shift on the environmental axis (bleaching response c-d to x-z; symbiosis response a-b to q-s). The effect of this is more growth (later shut-down of maximum growth at x) and higher resistance to environmental stress (bleaching and mortality at higher levels of external stress x-z vs c-d and q-s vs. a-b).

Linear shifts in the bleaching response thus result in high growth-high stress resistance (x-z/q-s) and low growth-low stress resistance (w-y/p-r) strategies. Applied to a hypothetical environment (Fig. 3), the linear shifts correspond to shifts between the paired induction/maximum thresholds A-C and B-D.

Non-linear shifts up or down the environmental axis, of induction and maximum bleaching thresholds result in changes in the slope of the bleaching response, i.e. a change in the counteractive capacity (Fig. 2b, Table 2). The simplest non-linear responses are:

- down-shift of the bleaching induction threshold (c to w) paired with no change or up-shift in the maximum threshold (stable at d, or d to z). The effect of this is less growth (earlier shut-down of maximum growth at w) but a broader counteractive capacity, w-d or w-z, and potentially higher resistance to environmental stress if the maximum threshold increases to z. The corresponding symbiosis shift is from a-b to p-b, or to p-s.
- up-shift of the bleaching induction threshold (c to x) paired with no change or down-shift in the maximum threshold (stable at d, or d to y). The effect of this is more growth (later shut-down of maximum growth from c to x) but a narrower counteractive capacity, x-d, and potentially less resistance to environmental stress if the maximum threshold is reduced, x-y. This reduction in the maximum threshold may happen as a result of higher stress due to greater internal metabolic imbalance at the higher environmental stress levels. The corresponding symbiosis response is p-b, or even p-r.

Non-linear shifts in the bleaching responses, result in high growth-low stress resistance (x-y/p-r) and low growth-high stress resistance (w-z/q-s) strategies. Applied to a hypothetical environment, the non-linear shifts correspond to paired induction/maximum thresholds A-D and B-C (Fig. 3).

Other non-linear shifts may be possible, however these can all be expressed as variants on the above two options based on whether the bleaching response curves intersect (basic non-linear response above) or not (basic linear response above).

Discussion

The selective pressures that result in non-linear shifts can be described as follows. A shift in the induction threshold for bleaching, from c to w or x will result in earlier and later induction of bleaching (respectively). If environmental stress stays within that range, then a species with threshold x will always outcompete a species with threshold w, as all other things being equal, growth of species “x” will be unimpaired by bleaching, while growth of species “w” will decrease due to lower zooxanthellae density and production once c has been crossed. If there is no change in the maximum threshold

for bleaching, d, then species x-d will always outcompete species w-d. This effect would be increased if a linear shift (Fig. 2a) were to occur (i.e. x-y and x-z, respectively). Such shifts are incompatible with life history theory as tradeoffs in resource allocation prevent positive correlation between high growth and stress resistance (Stearns 1989, Hoffman and Parsons 1989). In any community if there is a species or genotype that could grow fastest and always resist stress, it would always dominate.

Tradeoffs imposed by life history options require that shifts in the bleaching induction threshold up or down are complemented by an opposite shift in the maximum thresholds, such that the species with induction threshold x will tend towards maximum threshold y and species with induction threshold w will tend towards maximum threshold z. This allows for a change in competitive advantage between the species once the environmental stress has exceeded the point at which the bleaching responses intersect (Fig. 2b). Below the intersection the fast-growing species x-y will dominate, but above the intersection the stress resistant species w-z will dominate.

Growth/production maximizers (x-y/p-r)

These species will tend to maximize utilization by the host of fixed products from the zooxanthellae and may also include maximization of production by zooxanthellae. They are dependent on their high growth (or reproductive) rate for competitive success and extension over space. Their tradeoff is a high opportunity cost of reducing production. Thus they may adapt to raise the induction threshold of their bleaching response to postpone decreased production. If conditions deteriorate further, bleaching will occur, and it is possible that tissue damage may be higher as a result of the raised induction threshold and higher levels of internal metabolic stress. This raised internal stress may narrow the counteractive capacity, effectively lowering the maximum threshold and bringing on earlier collapse of the symbiosis.

In a model environment, growth/production maximizers will have induction and maximum bleaching thresholds at B and C, respectively (Fig. 3). If environmental conditions stabilize before the induction threshold (E1), or perhaps at mid-levels in the bleaching response, the strategy is successful. However the lowered maximum threshold means they are more vulnerable where conditions do not improve or in highly fluctuating environments (E2) so have a high risk of mortality. These species thrive and dominate space in habitat E1, outcompeting other species, but suffer high mortality in E2.

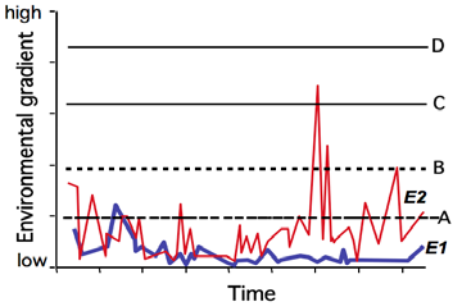

Figure 3. Environmental variability in benign (E1) and extreme (E2) habitats over time. Induction (horizontal lines A, B) and maximum (C, D) thresholds of a bleaching stress response (see Fig. 2) are shown. See discussion for details.

Stress tolerators/persisters (w-z/q-s)

These species will tend to minimize overall metabolism and therefore also production by zooxanthellae, and emphasize efficient use or management of fixed products by the host. They are dependent on their ability to persist under adverse conditions to maintain their space. Their tradeoff is low performance under good conditions. With a low opportunity cost of reducing production, early response to stress may develop. If this decreases the buildup of internal stress it may have the effect of broadening the range over which bleaching occurs, raising the maximum threshold that can be tolerated. This will further broaden the counteractive capacity and delay collapse of the symbiosis.

In a model environment, stress tolerators will have induction and maximum bleaching thresholds at A and D, respectively (Fig. 3). Under consistently benign conditions (E1), they cannot compete with production maximizers as they grow slower, and suppress growth at lower environmental stress levels. However, their higher maximum thresholds mean the can persist in widely fluctuating habitats (E2), with a low risk of mortality. These species persist in habitat E2, but are outcompeted by other species in E1.

These strategy descriptions fit classical life history strategies, both for r/K strategies and C-S-R strategies (Table 1), with the caveat that competitive and opportunistic strategies are both high growth/high risk strategies, but are optimized to different environmental conditions. With respect to bleaching and thermal stress, differences between ‘winners and losers’ have been described (e.g. Loya et al. 2001), explained by aspects of gross morphology, such as colony shape and tissue thickness. The analysis here offers an alternative framework based on metabolic rates and growth/resistance strategy, and further research may be able to show if growth form and other variables may be correlates or secondary to these. Certainly growth form

Table 3. Bleaching strategies summarized from Obura (2001) applied to life history strategies discussed in this paper.

Bleaching strategy	High bleaching/high growth	Variable bleaching/mortality	Persistent bleaching/low mortality
Bleaching observations	Severe bleaching response followed by near-100% mortality. Low stress resistance. Species generally fast growing and opportunistic. *Acropora, Pocillopora*	Graduated bleaching response with pale tissue, moderate mortality and recovery. Moderate to high stress resistance. Species generally slow growing, some large.	Persistent bleaching, low mortality. Moderate to high stress resistance. *Porites, Turbinaria, Astreopora*.
Life history strategy	Competitive and opportunistic	Intermediate/variable	Stress/adversity resistance

is a function of growth rates, where rapid growth is only possible for high surface area:volume growth forms such as branches and thin plates, and reproductive output is a direct function of capacity for photosynthetic output, egg production and tissue area. Branching corals are clearly the most susceptible to severe bleaching and to mortality following bleaching (Table 3) (Loya et al. 2001, Obura 2001, McClanahan 2004), while slow growing massives of both small and large sizes are the most stress resistant species (Table 1). Other variables such as tissue thickness are less clear in this regard, and further research may reveal a link.

In building up evidence for or against this hypothesis, the considerable variability in bleaching responses, and in the first-order responses that may dramatically alter the induction and appearance of bleaching, must be carefully considered. For example, a straightforward interpretation would state that stress tolerant corals would bleach earlier but more mildly than fast growing corals (Figs. 2 & 3). This can be seen in some cases of seasonal bleaching where small and large *Porites* massive colonies in lagoon reefs in East Africa often bleach at temperatures in the local spring (rapidly rising but intermediate temperatures in November) that don't cause bleaching in more susceptible species (pers. obs.). But during local-summer bleaching the first corals to bleach are usually the competitive and opportunistic genera. As expected though, they do tend to bleach more severely (steep response curve) and suffer mortality early. It may be that many of the pre-bleaching stress responses (Obura 2009) in stress tolerant corals are also tuned to an adversity response such that they effectively minimize internal stress thus pushing the bleaching induction threshold farther up the stress gradient than suggested by Figs. 2b and 3 with a combined effect of raising the maximum bleaching threshold.

The complex interactions and multiple stress responses in coral-zooxanthellae symbiosis (Brown 1997) contribute to variability in observed responses and explanations of bleaching (Coles and Brown 2003, Hoegh-Guldberg 2005) and a broad phase-space of bleaching-mortality dynamics (McClanahan 2004). As noted by Douglas (2003) this argues against overly-deterministic attempts to explain bleaching in terms of single phenomena. This life history model based on whole-organism emergent behaviour (Obura 2009) helps to identify patterns of acclimatization and adaptation above the detail of individual mechanisms that are precursors to the bleaching response itself.\

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Protracted declines in coral cover and fish abundance following climate-induced coral bleaching on the Great Barrier Reef

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Abstract. Understanding how corals and coral-dependant organisms respond to major disturbances is critical in predicting long-term changes in the structure and dynamics of coral reef assemblages affected by ongoing climate change. This study documents changes in coral and butterflyfish assemblages at Trunk Reef in the central Great Barrier Reef, Australia, following severe climate-induced coral bleaching in 2001-02. Coral cover declined by 90%, to a low of 3.2% (± 0.8SE) in March 2005. However, coral cover has started to recover, increasing to 6.5% in January 2008 (±1.0 SE). Despite recent increases in the abundance of corals there has been no apparent increase in abundance of butterflyfishes. If anything, overall densities of butterflyfishes declined even further between 2005 and 2008, due to recent declines in the abundance of non-coral feeding butterfly fish. Although there are some signs of coral recovery, it is clear that severe episodes of coral bleaching can have enduring effects on coral reef ecosystems, and that recovery typically takes many years (>5 years). Protracted declines and limited recovery of coral and fish communities indicate that reef ecosystems will gradually deteriorate as bleaching events become more frequent and more severe.

Key words: Bleaching, Butterflyfishes, Coral Reefs, Coral loss, Disturbance, Reef fishes

Introduction

Coral reef ecosystems are subject to frequent and often catastrophic disturbances caused by a variety of different agents, including severe tropical storms, freshwater plumes, temperature extremes, and infestations of the coral eating crown-of-thorns sea star. These acute, but increasingly frequent, disturbances often cause marked reductions in the abundance of reef-building corals (Hughes et al. 2003; Hoegh-Guldberg et al. 2007), which are the major architects and significant contributors to endogenous carbon production on coral reefs. Changes in the physical and biological structure of benthic reef habitats are likely to have further, often detrimental, effects on other reef associated organisms, particularly coral reef fishes (Wilson et al, 2006; Pratchett et al. 2008a).

Among those fishes with the greatest reliance on hard corals are butterflyfishes from the genus Chaetodon (family Chaetodontidae), many of which feed on hard corals (Harmelin-Vivien and Bouchon-Navaro 1983; Halford et al. 2004; Pratchett 2005). Spatial variation in the abundance of butterflyfishes is often strongly correlated with hard coral cover (Cadoret et al. 1999; Pratchett and Berumen 2008), indicating a close association between butterflyfishes and coral prey. Moreover, many butterflyfishes exhibit rapid and dramatic declines in abundance following coral depletion (Williams 1986; Pratchett et al. 2006; Graham 2007).

The strong reliance on corals by coral-feeding butterflyfishes makes them very susceptible to effects of climate-induced coral bleaching (Pratchett et al, 2006). However, provided bleaching is sporadic and does not cause 100% mortality of critical coral species, even highly susceptible fish populations may have the potential to persist and recover. The recovery and resilience of fishes will however, be critically dependant upon recovery of coral habitats (Halford et al. 2004). In the absence of any further disturbances, it may take as little as 5 years for coral cover to return to pre-disturbance levels (Halford et al. 2004), though this will depend on the severity and spatial extent of coral loss, which dictates the ability of surviving corals to recover, reproduce and reseed affected areas (Hughes and Connell 1999; Riegl and Piller 2003; Golbuu et al. 2007). Recovery will be much faster if at least some coral colonies survive the bleaching (Baird and Marshall 2002), because growth of surviving corals leads to more rapid increases in coral cover compared with settlement and subsequent growth of new individuals (Connell et al. 1997).

The purpose of this study was measure rates of recovery in butterflyfish and coral assemblages at Trunk reef, Great Barrier Reef (GBR), Australia, following climate-induced coral bleaching in 2001-

02. Coral depletion was further exacerbated by localised outbreaks of *Acanthaster planci* causing extensive and widespread coral loss throughout the region (Sweatman et al. 2004). Periodic monitoring of butterflyfish and coral assemblages have been undertaken at Trunk Reef since May 2000, testing for long-term changes in abundance of both fish and corals. Until 2005, both coral cover and abundance of most butterflyfishes had exhibited significant declines (Pratchett et al. 2006). It was expected however, that coral cover would have increased significantly from 2005 to 2008, and that coral recovery would initiate commensurate increases in the abundance of coral-dependant butterflyfishes, following Halford et al. (2004).

Material and Methods

This study was conducted at Trunk Reef (18°17'S, 146°53'E), in the central section of the Great Barrier Reef (GBR), Australia. Trunk reef is a large (ca. 125km^2) submerged reef, located ~120km north of Townsville. This reef, like many throughout the GBR, was subject to extensive and wide-spread coral bleaching during the summer (November – March) of 2001-02 (Berkelmans et al. 2004). In order to assess impacts of coral bleaching, local assemblages of both scleractinian corals and butterflyfishes were examined at Trunk Reef in May 2000 (18 months before the bleaching), in March 2002 (towards the end of the bleaching event), in March 2005 (3 years after the bleaching), and most recently, in January 2008 (>5 years post-bleaching). Sampling was conducted on the shallow reef crest (2-3m water depth), at three randomly selected sites along the exposed (south-east) side of Trunk Reef. All sites were non-overlapping and independent, but were very similar in their physical structure, aspect, and exposure to prevailing south-east trade winds.

In 2000, 2002, and 2005, coral cover and composition were assessed using replicate 10-m line-intercept transects. Ten replicate transects were sampled at each site. On each transect, colonies lying directly beneath the transect tape were identified to genus and the intercept length measured to the nearest centimeter. Percentage cover for each taxa was then calculated based on the cumulative intercept length of each taxa on each transect. In 2008 however, coral cover and composition was quantified using photographic records of the benthos taken at 1-m intervals along the length of 50-m transects that were deployed to survey butterflyfishes. Within each photograph we recorded the substrate type or benthic organisms immediately beneath the transect line corresponding with 1-m markings (e.g., 1.0, 2.0 etc).

Densities of butterflyfishes on the reef crest at Trunk reef were quantified using underwater visual census (UVC) along 50-m transects. Adult butterflyfishes observed within 2-m either side of the transect line were counted and recorded to species. All transects were positioned within 10-m of the edge of the reef crest, following the natural contours of the reef. Five replicate transects were conducted at each site. To test for differences in responses of butterflyfishes according to their specific trophic requirements (following Pratchett et al. 2006; Graham 2007), all species were categorised as either i) obligate corallivores (OC), ii) facultative corallivores (FC), or iii) non-coral feeders (NC). Species were assigned to feeding guilds following Pratchett (2005).

Results

Coral cover on the reef crest at Trunk reef declined significantly between May 2000 and March 2005, to a low of 3.2% (± 0.8SE) in March 2005 (Fig. 1, Table 1). Since 2005, there has been a 100% increase in coral cover to 6.5% (±1.0 SE) in January 2008 (Fig. 1). Recent increases in coral cover are mainly due to increased cover of *Acropora* species (Table 1), especially *A. hyacinthus*. In January 2008, mean cover of *Acropora* spp. was 4.8%, accounting for 73% of live coral cover, compared to 14% and 4%, for the next most abundant genera, *Pocillopora* and *Porites*, respectively. Other genera (comprising mainly *Favites*, *Goniastrea*, *Montipora*, and *Stylophora*) together accounted for only 8% of coral recorded in 2008.

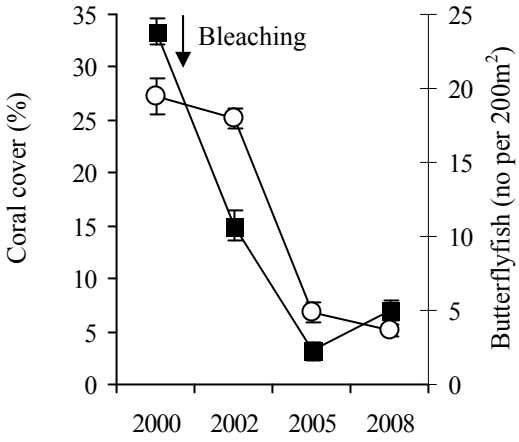

Figure 1. Mean ± SE percentage cover of scleractinian corals (■) and densities of butterflyfishes (O) at Trunk Reef in May 2000 (before bleaching), March 2002 (during bleaching), March 2005, and January 2008 (post-bleaching).

Table 1. Changes (%) in coral cover and butterflyfish abundance at Trunk Reef over two distinct intervals (2000-2005, and 2005-2008) Absolute changes were analysed using nested ANOVAs (between years and among sites within years), with F and p shown for temporal contrasts. Coral cover data was arcsine- square root transformed, while butterflyfish densities were $\log_{10}$ transformed prior to analyses (df = 1, 4). Values in bold are significant even when applying Bonferroni correction for multiple comparisons across coral genera and trophic groups for butterflyfishes (OC – Obligate corallivores, FC – Facultative corallivores, and NC – Non-coral feeders).

	2000-2005		2005-2008	
	Change	F, *p*	Change	F, *p*
Coral Cover	-90.4%	**236.4, 0.00**	104.0%	**8.96, 0.03**
Acropora	-85.8%	**87.9, 0.00**	207.0%	**10.7, 0.02**
Pocillopora	-50.1%	2.89, 0.16	-16.7%	0.02, 0.88
Porites	-96.9%	**27.6, 0.01**	9.6%	0.74, 0.43
Others	-97.8%	**114.9, 0.00**	90.5%	3.08, 0.14
Butterflyfish	-75.0%	**57.7, 0.00**	-24.7%	1.61, 0.27
OC	-90.8%	**172.8, 0.00**	-9.5%	0.20, 0.68
FC	-29.8%	1.18, 0.34	-24.2%	1.07, 0.36
NC	18.8%	0.09, 0.77	-47.5%	**57.4, 0.00**

Despite recent increases in coral cover at Trunk Reef, there has not been any concomitant increase in the abundance of butterflyfishes (Fig. 1). Mean densities of butterflyfishes declined from 19.5 (±1.2SE) fishes per 200m^2 (per transect) in March 2000 down to 3.7 (±0.4SE) fishes per 200m^2 in January 2008. The greatest decline occurred between 2002 and 2005, but there have been further (albeit not significant) declines between 2005 and 2008 (Fig. 1).

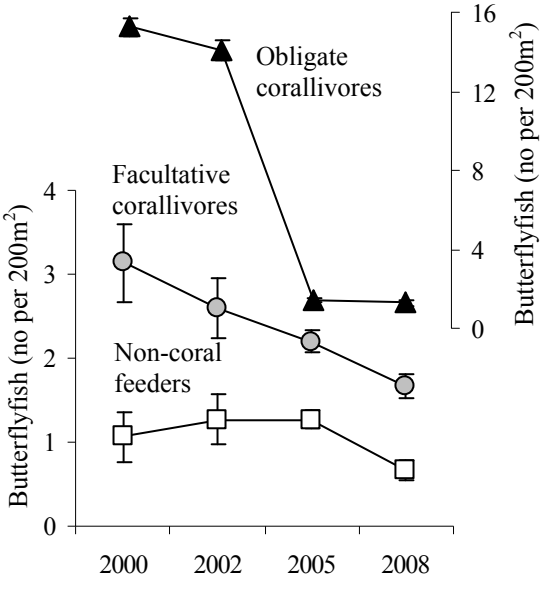

Figure 2. Temporal variation in mean ± SE densities of butterflyfishes within each trophic group (i. obligate corallivores, ii. facultative corallivores, and iii. non-coral feeders) on the reef crest at Trunk Reef

The group of butterflyfishes that exhibited the most rapid and dramatic declines in abundance following extensive coral depletion were the obligate coral feeders (comprising *Chaetodon aureofasciatus, C. baronessa, C. lunulatus, C. plebeius, C. rainfordi,* and *C. trifascialis*). Between 2002 and 2005, the proportional decline in abundance of these species corresponded very closely to the decline in total coral cover (Table 1). Densities of obligate corallivores have remained at or below 1.4 (±0.8SE) fishes per 200m^2 since 2005 with no evidence of recovery (Table 1).

Facultative corallivores (e.g., *C. citrinellus* and *C. melannotus*) and non-coral feeding butterflyfishes (e.g,, *C. auriga* and *C. vagabundus*) did not exhibit any significant variation in abundance to 2005 (Table 1), though there was an apparent downward trend in mean densities of facultative corallivores throughout this study (Fig. 2). For non-coral feeders, there was no apparent change in abundance between 2000 and 2005, but there have been significant recent declines in abundance (Table 1), from 1.3 (±0.1SE) fishes per transect in 2005 down to 0.7 (±0.1SE) in 2008 (Fig. 2).

Discussion

Climate-induced coral bleaching represents one of the most significant and increasingly prevalent disturbances to coral reef ecosystems, which not only causes extensive coral mortality, but also reduces the abundance of many other coral reef organisms that are reliant on corals for food, shelter or recruitment (Wilson et al, 2006; Pratchett et al, 2008a). At Trunk Reef, extensive coral depletion was accompanied by a 5-fold reduction in the abundance of butterflyfishes. Declines in abundance of butterflyfishes were most rapid and most severe for obligate corallivores, which is to be expected given their obligate dependence on coral for food. Corallivorous fishes have the most apparent and direct reliance on live corals and are consistently among the worst affected fishes following extensive coral loss (Wilson et al. 2006).

The extent to which corallivorous fishes feed on corals (versus other non-coral prey) is highly variable (e.g., Hobson, 1974; Pratchett, 2005) and obligate coral-feeders are much more affected by coral loss than facultative coral-feeders (Bouchon-Navaro et al. 1985; Williams 1986; Pratchett et al. 2006; Graham 2007). Accordingly, facultative corallivores were relatively unaffected by initial declines in abundance of corals at Trunk Reef. These fishes presumably persisted on increased intake of non-coral prey, but there has been a persistent decline in abundance of these fishes throughout the study, suggesting that corals may represent an important and necessary component of their diet. Alternatively, protracted

declines in the abundance of these fishes may be attributable to the loss of habitat structure or habitat diversity associated with extensive coral loss (Graham et al. 2006; Pratchett et al. 2008a), and this may also be the reason why non-coral feeding butterflyfishes have ultimately declined in abundance >5 years after the initial coral loss. It is also possible that coral depletion has reduced settlement success by butterflyfishes (Jones et al. 2004), which might explain protracted declines in population size. However, butterflyfishes with low coral content in their diet as adults tend not to recruit to live corals (Pratchett et al. 2008b), but overall declines in habitat complexity may have reduced post-settlement survivorship of all species (Graham et al. 2007).

Topographic complexity of coral reef habitats has an important influence on biotic interactions, such as predation and competition, and has a major influence on the local abundance of coral-reef fishes, especially during early life stages (Almany 2004; Hixon and Jones 2005). Climate-induced coral bleaching kills corals, but leaves the underlying skeleton completely intact (Hoegh-Guldberg 1999). Exposed coral skeletons are then subject to a whole suite of bio-eroding organisms that undermine the structural integrity of these carbonate structures (Hutchings 1986). Reef habitats with reduced topographic complexity typically support lower fish abundance, fewer species, and increased evenness (Gratwicke and Speight 2005; Graham et al. 2006).

This study focused on specific study locations along the exposed reef crest at Trunk reef as baseline data on coral and butterflyfish assemblages were recorded prior to the bleaching in 2000 (Pratchett et al. 2004, 2006). However, the extent of coral depletion and declines in abundance of butterflyfishes (especially corallivorous species) reported for Trunk Reef are similar across the full range of locations (reef crest and reef slope habitat at three different reefs; Trunk Reef, Bramble Reef and Rib Reef) surveyed since 2002. Interestingly however, coral recovery has been highly variable within and among reefs. At Bramble reef, for example, there are two sites where coral cover has increased to 90% within the 5 years since the bleaching (Fig. 3). Even so, there has been no apparent recovery of butterflyfishes at any of these sites. These findings contradict previous studies that suggested recovery of fishes would closely follow coral recovery (Halford et al. 2004). Recovery of fish populations at these sites appears to be currently limited by a lack of new recruits. Recruitment rates by butterflyfishes are generally low (Pratchett et al. 2008b) and may have been further constrained by limited coral cover at potential settlement sites, as well as widespread depression of breeding populations.

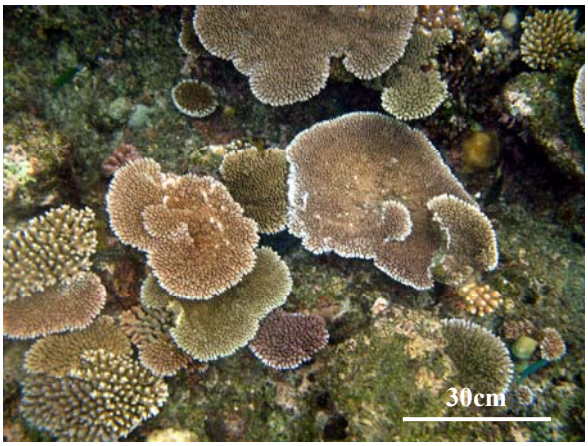

Figure 3. Extensive cover of *Acropora* (mostly, *A. hyacinthus*) at Bramble Reef in January 2008.

The rapid recovery and increasing dominance of *Acropora* spp. in the aftermath of extensive coral bleaching suggests that climate forcing of coral communities may initially favor coral species with rapid recovery potential, rather than slow-growing corals that might otherwise have greater resistance to bleaching, as suggested previously (Hughes et al. 2003; McClanahan et al, 2007). Fortunately, faster growing corals (especially *Acropora*) contribute most to topographic complexity of reef habitats (Sheppard et al. 2002) and are also the major corals used by corallivorous and coral-dwelling fishes (Munday et al. 1997; Pratchett 2005). However, persistence of *Acropora* populations will depend on sufficient time between successive bleaching events to allow for recovery. Recovery was mostly very limited (but occasionally spectacular) in the 5 years since extensive bleaching in the central GBR. Donner et al. (2005) project that bleaching is likely to occur at least every 2 years on most coral reefs around the world by 2040, meaning that there would be insufficient time for recovery of *Acropora* spp., let alone other slower growing species (e.g., *Porites*). Sustained and ongoing climate change will undoubtedly change the community structure of coral assemblages (Hughes et al. 2003; McClanahan et al. 2007), yet specific effects will depend on the severity, extent and recurrence of future bleaching events.

In conclusion, this study shows that coral communities are starting to recover at Trunk Reef. However, recovery has been very slow and follows highly protracted declines in coral cover. Moreover, coral depletion led to extensive and long-term declines in the abundance of butterflyfishes, and these fishes are yet to respond to increased availability of corals, which are critical in providing both food and habitat (Pratchett et al. 2006). These systems are likely to recover in the absence of any further

disturbances. However, it is clear that projected increases in the frequency and severity of coral-bleaching events are likely to cause extensive ongoing degradation and biodiversity loss on coral reefs throughout the world (Hoegh-Guldberg et al. 2007).

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Coral ultrastructural response to elevated pCO_2 and nutrients during tissue repair and regeneration

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Abstract. Corals and coral reefs have recently experienced widespread decline attributed to anthropogenic pressure on reef systems. Studies have demonstrated that nutrient and pCO_2 stress effect coral growth and calcification, but study of specific effects on coral tissue is lacking. The objective of this research was to examine wound healing in corals and how it is affected by exposure to elevated nutrients and pCO_2. Coral tissue repair and regeneration during wound healing in *Montastraea cavernosa* and *Porites astreoides* were assessed histologically and ultrastructurally by examining colony fragments exposed to elevated nitrate, phosphate, and pCO_2. In *M. cavernosa*, tissue repair was facilitated by granular amoebocytes, and the zooxanthellae population size increased under enriched nutrient conditions. In *P. astreoides,* zooxanthellae chloroplasts were markedly abnormal in phosphate-enriched corals, and the concentration of chromophore cells at the healing tissue front was markedly lower under elevated nutrient conditions. The area of wound healed was higher after 14 days under every experimental condition in *M. cavernosa* compared to *P. astreoides*. In both species, phosphate enrichment had the most deleterious effect on repair and regeneration.

Key words: coral ultrastructure, tissue repair, pCO_2, nutrient enrichment.

Introduction

A growing global population and the close proximity of coral reefs to coastal areas has resulted in increasing anthropogenic pressure on reef systems. In recent history, the implications of environmental change on coral reefs have become progressively more far-reaching. Both nutrification and global climate change (including increasing atmospheric pCO_2 and temperature) are sources of nonspecific general anthropogenic stress to corals. Other sources, such as over-fishing, sedimentation and turbidity from dredging and beach restoration activities, have deleterious effects on corals (Szmant 2002, Vargas-Ángel et al. 2005). In addition, direct physical damage can result from storms, coral collection, dynamite fishing, blasting, and ship groundings (Curtis 1985, Szmant 2002). Physical damage events, coupled with existing eutrophication stress (a complex process of organic production and accumulation) and changing global climate present a poor outlook for successful natural recovery of reef communities and individual colonies (Szmant 2002).

Elevated nutrient and pCO_2 levels in areas prone to physical damage may contribute to a reduced ability of damaged corals to successfully heal and survive. Significant decreases in calcification rate and/or growth rate have been observed in several species at nitrate concentrations of <5 μM (Tomascik and Sander 1985, Bell and Tomascik 1993, Marubini and Davies 1996, Renegar and Riegl 2005) and at phosphorus concentrations of >1 μM (Kinsey and Davies 1979, Walker and Ormond 1982, Tomascik and Sander 1985, Ferrier-Pagès et al. 2000, Renegar and Riegl 2005). Increasing atmospheric CO_2 partial pressure (pCO_2) is predicted to alter ocean surface carbonate saturation, resulting in reduced reef growth (Leclercq et al. 2000, Guinotte et al. 2003, Hughes et al. 2003). The possible effects of low pH and CO_3^{2-} (including weaker skeletons and increased erosion) may have a greater impact on net calcification than nutrient enrichment (Kleypas et al. 1999, Marubini and Atkinson 1999).

The process by which tissue repair takes place and normal function restored is a complex process that has been described in other calcifying aquatic organisms but remains largely uninvestigated in scleractinians. The mechanism of invertebrate tissue repair, organic matrix production and skeletal deposition has been ultrastructurally studied, for example, in echinoderms and molluscs (Wilbur 1973, Meenakshi et al. 1975, Blackwelder and Watabe 1977). Mollusc epithelial ultrastructure changes dramatically during repair, and the minerals formed differ from normal morphology and mineralogy (Watabe and Blackwelder 1980). In corals, most studies have focused on physical parameters such as lesion or colony size. Lesional

perimeter length is likely the most important factor in regeneration, although the size, shape and location of lesions can be significant (Meesters et al. 1997, Oren et al. 1998, Lirman 2000). Regeneration may be supported by a limited amount of energy related to the extent of damage, an aspect possibly linked to colony size (Bak and Van Es 1980, Lirman 2000).

A more complete understanding of the effects of anthropogenic environmental factors on coral cell biology is essential to reef management and prediction of the capacity for natural recovery. This goal of this study was to examine the process of tissue repair in corals and how it is affected by elevated nutrients and pCO_2. The target species, *Montastraea cavernosa* and *Porites astreoides*, are important and widespread Caribbean reef corals.

Materials and Methods

Four colonies each of *M. cavernosa* and *P. astreoides* were acclimated to laboratory conditions. Colonies were cut into 4 cm^2 fragments. A wound (~4 mm wide and 2 cm in length) was created with a rotary tool in each fragment, and fragments were placed in experimental tanks. Experiments were conducted in 20 separate (8 l) flow-through aquaria partially submerged in a water bath to control temperature variation between tanks. Five treatment conditions were maintained, with two tanks and 32 fragments of each species for each treatment: control; nitrate enrichment; phosphate enrichment; nitrate and phosphate, and pCO_2 enrichment. Each set of treatment tanks was continuously supplied with natural seawater from reservoirs dosed at a specific rate. Irradiance was supplied by metal halide lamps (175 watt, 10,000K, photoperiod 12:12).

Elevated mean nutrient concentrations of 10.8 (±0.5) µM NO_3^- and 4.4 (±0.3) µM $P\text{-}PO_4^{3-}$ were achieved by addition of KNO_3 and KH_2PO_4 to reservoirs supplying the appropriate tanks. Nitrate concentration was determined with NECi Saltwater Nitrate Test Kit (SW-NTK). Phosphate concentration was determined utilizing the method of Parsons et al. (1984). Elevated mean pCO_2 concentrations of 1381 (±66) µatm were achieved with a pH controlled CO_2 injected reservoir system described by Reynaud et al (2003). Total alkalinity and pH were used to monitor pCO_2.

Corals were maintained under experimental conditions for 14 days. Fragments were then fixed in glutaraldehyde fixative solution [2 mL 70% glutaraldehyde in 68 mL cacodylic buffer (2.16 g cacodylic acid in 200 mL of .22 µm filtered seawater)]. Samples were maintained at 4°C in the fixative solution for 1-2 days, rinsed in buffer, and subsequently post-fixed in buffered 1% osmium tetroxide solution (5 mL 4% aqueous osmium tetroxide in 30 mL of cacodylic buffer) for 1 hour. Samples were again rinsed in buffer and then dehydrated in a graded series of ethanols. Excess skeleton was trimmed and the samples were embedded in Spurr resin. Ultrathin sections were cut (40 to 60 nm thick) using a Sorval MT-2 ultramicrotome fitted with a diamond knife. Sections were retrieved on nitrocellulose and carbon coated 200 mesh copper grids, stained with Reynolds lead citrate and/or 2% uranyl acetate solution, and viewed in a Phillips 300 TEM.

After fixation and dehydration as discussed above, SEM samples were dried in HMDS, mounted on carbon adhesive covered aluminum stubs, coated with palladium in a sputter coater and examined in an FEI XL-3- ESEM fitted with an Oxford EDS for elemental analysis of the calcified structures.

Histological samples were decalcified after primary fixation in 5% HCl/EDTA solution, dehydrated and embedded in Paraplast®, sectioned and stained with Hematoxylin & Eosin.

Results

Wound healing and closure was affected by nutrient and CO_2 enrichment. Area analysis of percent wound healing for each treatment was performed using Coral Point Count (Kohler and Gill 2006) (Table 1). In *M. cavernosa*, 15% of the wounded corals for all treatments fully healed after 14 days. In *P. astreoides*, none of the wounded corals fully healed. In both species, phosphate enrichment had the greatest deleterious effect on the percent of wound repaired. The effect of pCO_2 was similar to that of nitrate enrichment alone.

Table 1. CPCe (V 3.5). Mean percent wound healing in each treatment for *Montastraea cavernosa* and *Porites astreoides*.

	Montastraea cavernosa	***Porites astreoides***
Control	**87% ± 11%**	**24% ± 5%**
Nitrate/Phosphate	**85% ± 17%**	**9% ± 13%**
Nitrate	**79% ± 11%**	**10% ± 16%**
Phosphate	**58% ± 26%**	**2% ± 6%**
pCO_2	**75% ± 20%**	**10% ± 11%**

Montastraea cavernosa. Histological analysis indicated that tissue repair in *M. cavernosa* was characterized by rapid granulation of tissue across the wound site, facilitated by granular amoebocytes. These amoebocytes coalesced to form new tissue at the healing front (arrows) (Figs. 1A & 1C). No histological differences in the coral tissue have thus far been observed between the treatments. However, the zooxanthellae population number appeared to increase in response to nutrient enrichment (Figs. 1B & 1D).

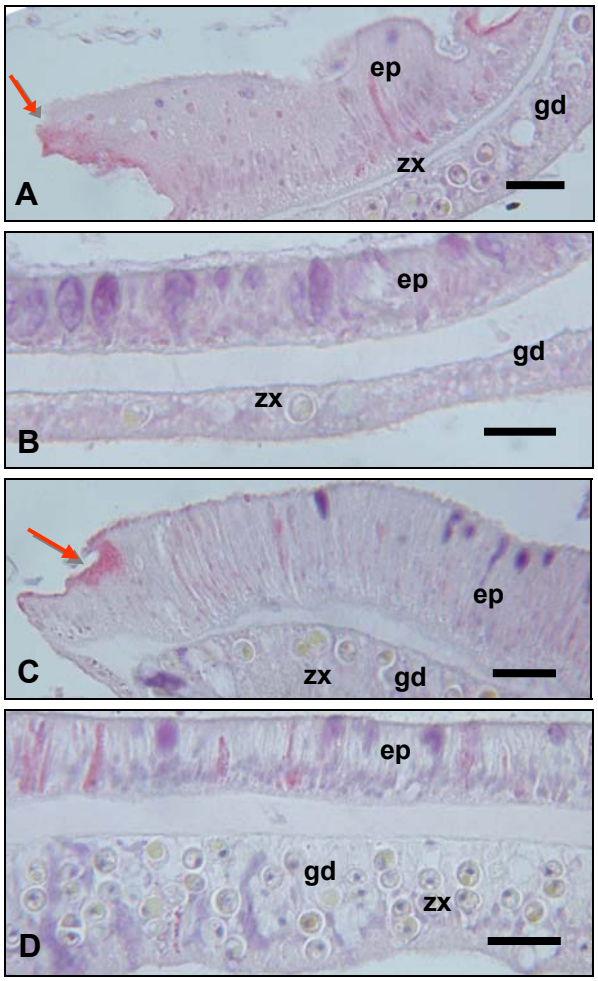

Figure 1. *Montastraea cavernosa*. Histological micrographs. A) Control, B) CO_2 enriched, C) nitrate & phosphate enriched and D) phosphate enriched. ep: epidermis; gd: gastrodermis; zx: zooxanthellae. Scale bars: A, B, C & D = 20 µm.

Ultrastructural observations revealed that granular amoebocytes were migrating to and integrating with new tissue at the repairing interface (Figs. 2A & 2B). Newly formed tissue was dense with well-defined cell walls and a distinct granular appearance (Fig. 2C). Zooxanthellae appeared healthy and were in various stages of cell division. Preliminary data indicates few distinct differences in tissue ultrastructure between nutrient, CO_2, or control treatments.

Porites astreoides. Tissue repair in *P. astreoides* was characterized by an increased concentration of chromophores near the healing tissue front (arrows). This was pronounced in control and CO_2 treatments (Figs. 3A & 3B). In contrast, the nutrient enriched corals appeared to exhibit fewer chromophores near the repairing front (Figs. 3C & 3D), and the gastrodermis was thickened. Vacuolization was observed in phosphate treated tissue, suggesting zooxanthellae degradation (Fig. 3D).

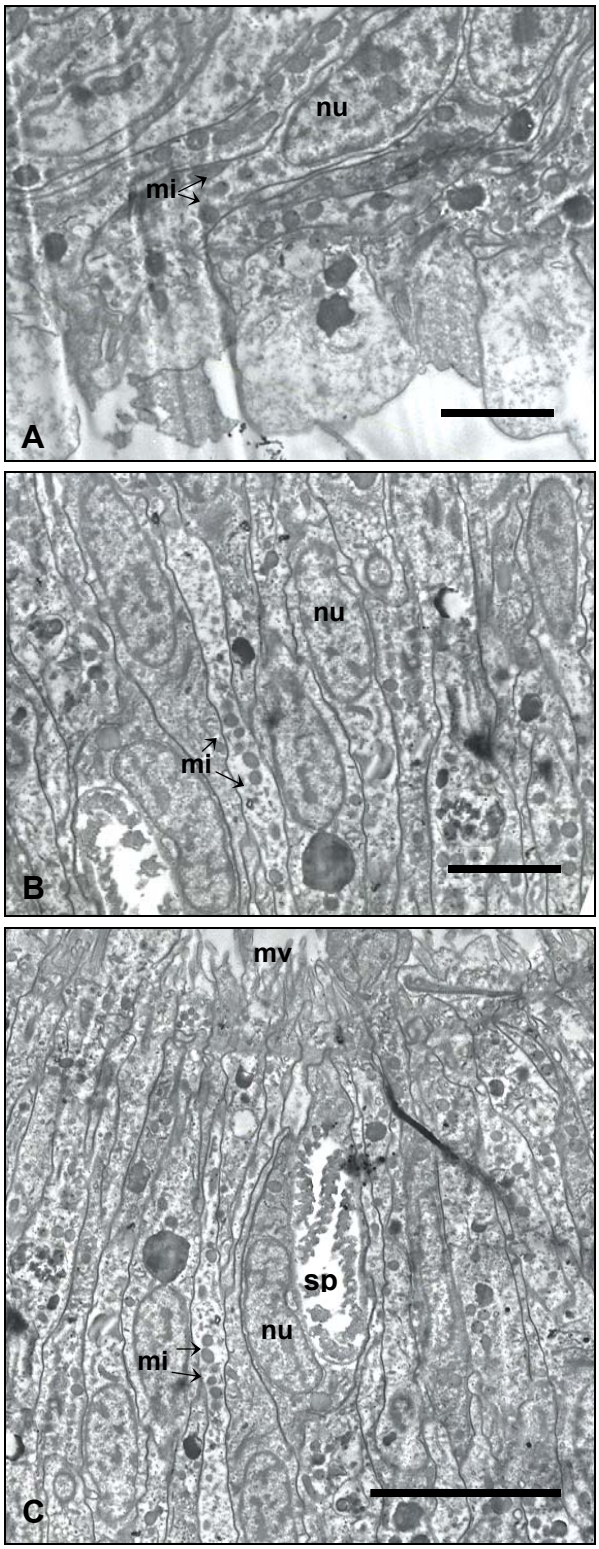

Figure 2. *Montastraea cavernosa*. TEM micrographs. A) Coalescent granular amoebocytes, B) mid-stage tissue formation, and C) late-stage tissue formation. mi: mitochondria; mv: microvilli; nu: nucleus; sp: spirocyst. Scale bars: A = 4 µm, B & C = 5 µm.

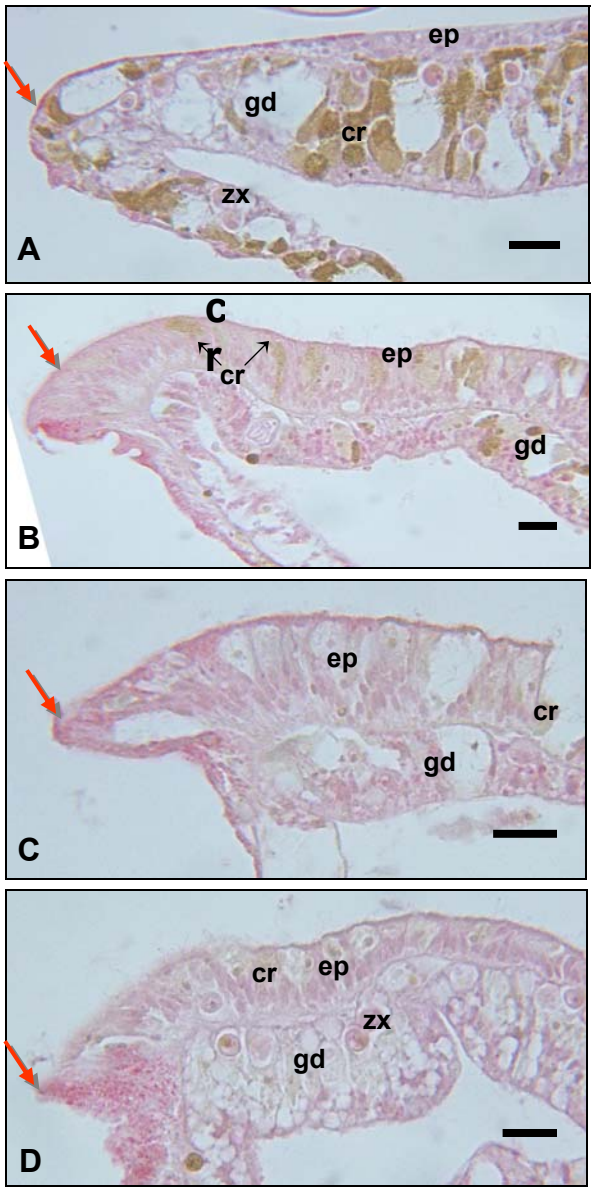

Figure 3. *Porites astreoides.* Histological micrographs. A) Control, B) CO_2 enriched, C) nitrate & phosphate enriched and D) phosphate enriched. cr: chromophore; ep: epidermis; gd: gastrodermis; zx: zooxanthellae. Scale bars: A, B, C & D = 20 µm.

Fine-structural examination (SEM) of the repairing tissue confirmed the presence of chromophores accumulating at the healing tissue front (oval) (Fig. 4A). Ultrastructural examination revealed mature granules within the chromophore cells (Fig. 4B). Abnormal zooxanthellae were observed in the phosphate-enriched corals, with significantly degraded chloroplast lamellae and cellular wall disruption (Fig. 4C). These effects are currently being assessed in greater detail.

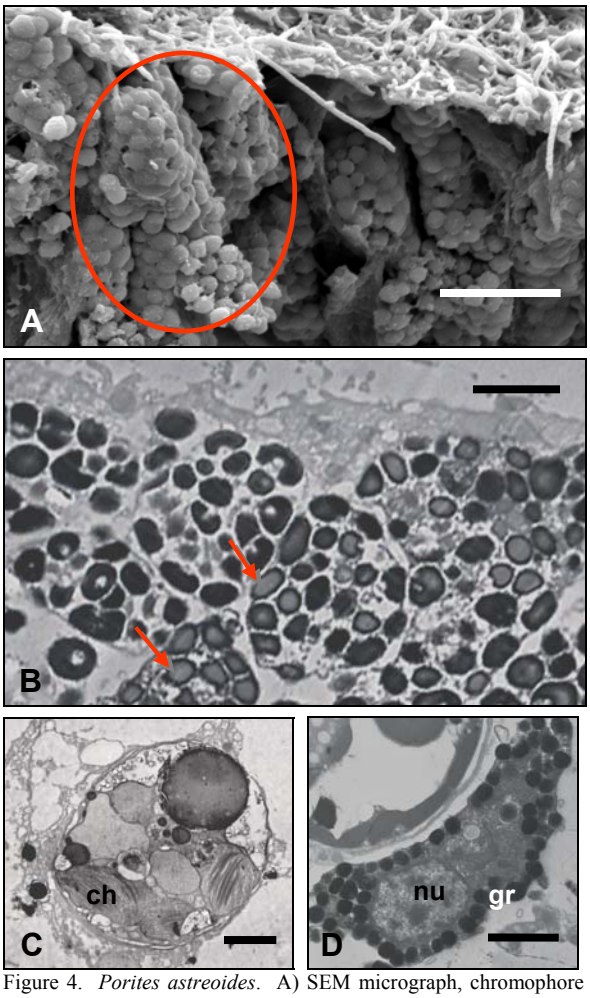

Figure 4. *Porites astreoides.* A) SEM micrograph, chromophore cell accumulation in healing tissue. TEM micrographs, B) mature granules in chromophores, C) phosphate-enriched zooxanthellae and D) mast-type cell in early stage tissue repair. ch: chloroplast; gr: granule; nu: nucleus. Scale bars: A = 5 µm, B, C & D = 2 µm.

Discussion

Tissue repair in *M. cavernosa* was characterized by granulation of new tissue across the wound site, facilitated by coalescent granular amoebocytes. This is similar to observations in gorgonians (Meszaros and Bigger 1999). As little organic matrix was observed associated with the calicodermis near the repairing front, the wound healing strategy of this species appears to emphasize rapid wound closure and formation of new tissue before calcification resumes. The percentage of wound repair was highest in the controls, and lowest in the phosphate treatment. Zooxanthellae concentration appeared higher near the healing front in the nutrient treatments compared to the control or CO_2 treatments. The variation in zooxanthellae concentration is likely due to the interaction between regeneration energy demands and the presence of limiting nutrients, as overall increases in zooxanthellae concentrations have been found in

wounded gorgonians compared to non-wounded (Meszaros and Bigger 1999). This aspect is currently being studied in greater detail.

In contrast to *M. cavernosa*, the wound repair strategy of *P. astreoides* appeared to involve rapid sealing and reorganization of the tissue and continuation of calcification, with closure achieved by recalcification across the wound. This is supported by the marked effect of nutrients and pCO_2 on wound closure rate in this species. Accumulation of chromophores at the healing tissue front was notable in the control and CO_2 treatments, in contrast to the few seen adjacent to the tissue front in the nutrient treatments. Interestingly, the ultrastructure of some chromophores was similar to vertebrate mast cells (Fig. 4D) (Porter and Bonneville 1974). Chromopphores may have many functions, including involvement in tissue repair and the coral immune response. For example, differences in chromophore ultrastructure have been observed in healthy vs. diseased *P. astreoides* (Kacszmarsky, pers. com.).

The synergistic effect of nitrate and phosphate combined appeared to be antagonistic in both species. Similar observations have been made regarding growth rate and calcification in other coral species. The effect of nitrate and phosphate combined on the growth rate of *Acropora cervicornis* was additive at low concentrations (5 μM NO_3^- and 2 μM P-PO_4), and antagonistic at high concentrations (10 μM NO_3^- and 4 μM P-PO_4) (Renegar and Riegl 2005). This effect may be a consequence of disruption of the coral-zooxanthellae relationship resulting from the stress of and energy requirements for regeneration and repair (Meszaros and Bigger 1999).

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Comparative investigation of organic matter release by corals and benthic reef algae – implications for pelagic and benthic microbial metabolism

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Abstract. Global climate change and direct anthropogenic stress factors lead to gradual replacement of hermatypic corals by benthic algae at many reef locations, a process which is commonly referred to as phase shift. Recent research showed that corals via the release of organic matter and concomitant effects on cycles of matter can act as engineers of reef ecosystems. There are strong indications that reef associated benthic algae do also affect reef ecosystem functioning via organic matter release, but relevant information is lacking. To gain a better understanding of the biogeochemical consequences such phase shifts may entail, a series of comparative studies with corals and algae was conducted in reefs of the Northern Red Sea during four seasonal expeditions in 2006-2008. These investigations focused on the quantity and quality of the organic matter released by both groups of organisms involving dissolved organic carbon (DOC), particulate organic carbon (POC) and nitrogen (PN) along with the respective stable isotope signatures. Planktonic and benthic degradation of the released material were investigated using bottle incubation experiments and in-situ stirred benthic chambers. First outcomes show clear differences between organic matter release by corals and algae, thus suggest effects of phase shifts onto reef biogeochemical cycles.

Key words: corals, reef algae, organic matter release, phase shift, community metabolism

Introduction

It is generally assumed, that the global climate change along with direct anthropogenic factors like eutrophication and overfishing lead to phase shifts in coral reefs, i.e. the gradual replacement of reef building corals by benthic algae (Hoegh-Guldberg 1999, Hughes et al. 2003, Pandolfi et al. 2005, Hoegh-Guldberg et al. 2007, Hughes et al. 2007). Recent studies also showed that hermatypic corals can act as engineers of the entire reef ecosystem, particularly by the release of organic matter and associated effects on biogeochemical key processes and element cycles (Wild et al. 2004a, Wild et al. 2005b, Wild et al. 2008). This is a newly discovered aspect of corals as ecosystem engineers besides their long known ability to generate structural frameworks.

Moreover, the work of Smith et al. (2006) indicates that benthic reef algae can also affect processes such as microbial activity in their surroundings via a hypothetical release of organic matter. Reef algae may therefore act as (new) reef ecosystem engineers, but likely in a very different way. This pilot study presents first data based on comparative investigations with the dominant corals and benthic reef algae from four expeditions to the Northern Red Sea comprising the following three interrelated approaches: 1) Quantification of dissolved and particulate organic matter (DOM and POM) release, 2) Determination of POM stable isotope signatures, 3) Planktonic and benthic degradation of released exudates. These data will provide first comparative information on the quantity and quality of benthic algae-derived organic matter and its subsequent degradation in the different compartments of the ecosystem coral reef.

Material and Methods

The work for this study was conducted during four seasonal expeditions (Nov/Dec 2006, Aug/Sep 2007, Feb/Mar 2008, May 2008) to Marine Science Station (MSS), Aqaba, Jordan. Collection of all specimens took place in the MSS fringing reef in water depths of 5 to 7 m. During each of the field trips, 5 replicate fragments (coral branch length: 6 to 10 cm) were broken off in-situ from colonies of the dominant hard corals of the genera *Acropora*, *Pocillopora* and *Stylophora*, which were allowed to heal in a flow-through aquarium for at least 7 d prior to the

subsequent experiments. In addition, 5 replicate small pieces (lengths: 6 to 14 cm) of the 3 most dominant types of benthic algae were collected in-situ: the green algae *Caulerpa* spec., the red algae *Peyssonnelia* spec., and typical filamentous turf algae consortia growing on dead coral skeletons. All algae were left in a flow-through aquarium for at least 12 h prior to the subsequent experiments for cleaning and healing purposes. For the organic matter release quantification the beaker incubation technique described by Herndl and Velimirov (1986) was used. Corals and benthic algae were separately transferred into acetone- and seawater-rinsed 1000 ml glass beakers filled with 800 to l000 ml of untreated seawater freshly pumped from the field. Identical beakers, only filled with seawater, served as controls. Beakers were kept in a flow-through aquarium during day at in-situ temperature of 21 to 29 °C (caused by seasonal differences) as monitored by *Onset HOBO* temperature loggers. Nylon gauze was clamped above the beakers to simulate light intensities very similar to those at 5 m water depth as verified by *Onset Pendant* light loggers. After 6 h incubation duration, corals and algae were removed from the beakers and sub-samples were taken from the incubation water for determination of the following parameters.

Dissolved Organic Carbon (DOC): Circa 10 ml of the incubation water were filtered through 0.2 µm sterile syringe filters (polyethersulfone membrane). The first 4 ml of the filtrate were discarded, but the following 6 ml were collected in pre-combusted brown glass bottles or ampoules, which were instantly frozen at -20 °C and kept frozen until analysis. DOC concentrations were determined by high temperature catalytic oxidation (HTCO) using a Rosemount Dohrmann DC-190 total organic carbon (TOC) analyser. After defrosting, each sample was treated by adding 100 µl of 20 % phosphoric acid and purging for 5 min in order to remove dissolved inorganic carbon. DOC concentration of each sample was measured five times. An outlier test was conducted and the DOC concentrations of the remaining samples were averaged. Potassium hydrogenphtalate was used as standard for calibrating the DC-190 TOC analyser.

Particulate Organic Carbon (POC) and Nitrogen (PN): Between 400 and 940 ml of the incubation water were filtered onto pre-combusted GF/F filters (Whatman, 25 mm diameter), which were dried for at least 48 h at 40 °C and kept dry until analysis. POC and PN concentration measurements and respective stable isotope analyses were performed with a Carlo Erba NC 2500 elemental analyzer, coupled with a THERMO/Finnigan Conflo II- interface to a THERMO/Finnigan MAT Delta plus isotope ratio mass spectrometer. Elemental concentrations were calculated from certified elemental standards (Atropine, Cyclohexanone-2,4-dinitrophenylhydrazone; Thermo Quest, Italy) and typically showed standard deviations < 3 %. Stable isotope ratios are given in the conventional delta notation ($\delta\ ^{13}C$, $\delta\ ^{15}N$) relative to Vienna PeeDee Belemnite (VPDB) standard (Craig 1957, Coplen 1995) and atmospheric nitrogen (Mariotti 1984), respectively. Standard deviations for repeated stable isotope measurements of lab standard (Peptone) were better than 0.15 ‰ for nitrogen and carbon, respectively. Respective surface areas of all coral fragments and algae pieces were measured as reference parameter using geometric approximations (all corals and turf algae growing on dead coral fragments, see Naumann et al. (2009) or the image analysis software *Image J* to analyze digital photographs of the predominantly 2-dimensionally growing macro algae *Caulerpa* spec. and *Peyssonnelia* spec..

Planktonic microbial degradation: Circa 140 ml of the incubation water from each beaker was used to fill two 60 ml gas-proof glass bottles. Oxygen concentration in one of the bottles was measured immediately and in the second bottle after incubation of the enclosed water for at least 16 h in the dark and at in-situ temperature using Winkler titration (Winkler 1888) or a *Hach HQ 10* optode. Microbial activity in the incubation water was determined by subtracting final from start oxygen concentration. Planktonic microbial degradation of the added TOC was calculated by using the respective POC + DOC amounts and the increase in O_2 consumption in the bottles relative to the controls assuming that 1 mol added organic material is oxidized by 1 mol O_2.

Benthic degradation: Degradation of algae and coral exudates was studied in-situ by addition of algae- and coral derived organic material to stirred benthic chambers identical to those described by Huettel and Gust (1992). These in-situ experiments were conducted at a reef site with carbonate sands (2.5 m water depth) described in (Wild et al. 2005a). The duration of the individual chamber experiments ranged between 5 to 8 h. Prior to each experiment, chambers were gently inserted into the loose calcareous sands to a depth of about 12 cm, thus including a water column of approximately 20 cm height and 5.7 l volume. At the beginning of the first experiment, 81 µmol coral- and 310 µmol algae-derived organic matters were added to two chambers each. In a second independent experiment, 91 µmol coral- and 186 µmol algae-derived organic matters were again added to two chambers each, but only one of these two replicate chambers was stirred (advection

chamber) , whereas the other one was left without stirring (diffusive chamber). All 8 chambers of both experiments were incubated for 8 h in the dark. Water samples were regularly (at least every 2 h) collected from all chambers through a sampling port using plastic syringes, whereby the water from the diffusive chambers was thoroughly mixed before sampling in order to avoid O_2 concentration gradients. Oxygen concentrations were measured in the chamber waters using Winkler titration and benthic TOC degradation of the added algae or coral exudates were calculated as described above.

Results

All investigated benthic reef algae released both DOM and POM in measurable quantities. Data from the first two seasonal expeditions showed that organic matter release by corals and benthic algae was very different. In particular, DOC fluxes were one order of magnitude higher during autumn 2006 compared to summer 2007 (Table 1). There was no correlation between organic matter release and water temperature. All investigated benthic reef algae during both seasons showed DOC release, whereas DOC release by the corals was highly variable (as indicated by the large error bars) with often negative values, i.e. DOC uptake (Table 1). POC release could be detected for all investigated specimens, but showed no seasonal differences with similar release rates in autumn and summer. However, corals generally released significantly more POC than algae (U-test after Wilcoxon, Mann and Whitney, $p < 0.05$). The C:N ratios and nitrogen stable isotope signatures of algae and coral-derived particulate organic matter (POM) were not significantly different, but carbon stable isotope signatures of algae-derived POM ($\delta\ ^{13}C$: -10.1 ± 1.4 ‰) were significantly more positive ($p < 0.05$) than those of coral-derived POM ($\delta\ ^{13}C$: -18.3 ± 0.3 ‰). POM C stable isotope signatures were very similar to that of sterile coral mucus ($\delta\ ^{13}C$: -18.2 ± 1.2 ‰; Naumann et al. unpublished data), thereby demonstrating the apparent dominance of this material in the coral beakers. The respirometric experiments from all 4 seasons revealed that microbial activity measured as O_2 consumption was only significantly higher in the algae incubation water compared to that of the corals in autumn, but not during the other three seasons. Resulting microbial Total Organic Carbon (TOC = POC + DOC) degradation rates in autumn were 0.57 ± 0.38 and 0.18 ± 0.02 % h^{-1} for the algae- and coral-derived exudates, respectively. Benthic degradation of both organic matter sources showed an opposite trend with twice as high TOC degradation rates for the added coral exudates (23.7 ± 4.8 % h^{-1}) than those for the algae exudates (12.1 ± 3.9 % h^{-1}) under advective conditions. Advective transport of matter induced by the stirred benthic chambers increased benthic C degradation by a factor of 8 for the coral exudates, but only doubled for the algae exudates.

Discussion

This study confirms that benthic reef algae similar to hermatypic corals release organic matter in dissolved and particulate form to their surrounding. The assumed differences in organic matter release between benthic reef algae and corals (please see introduction) are verified by the tendency that corals release more POC and algae more DOC as well as by the differences in carbon stable isotope signatures. The latter finding may be caused by a more pronounced photosynthetic C assimilation of the benthic reef algae (Fry 2006), but may also indicate different chemical composition of algae compared to coral exudates. This aspect needs further detailed chemical analyses, but the differences in natural C stable isotope signatures suggest the suitability of this material for natural tracer studies.

	Autumn 2006	
	DOC net release	POC net release
Turf	66.0 ± 23.0	2.7 ± 1.3
Caulerpa	10.0 ± 8.0	0.8 ± 0.2
Peyssonnelia	22.0 ± 18.0	2.2 ± 0.3
Acropora	105.0 ± 193.0	2.5 ± 0.6
Stylophora	-75.0 ± 45.0	7.8 ± 1.5
Pocillopora	-435.0 ± 30.0	2.8 ± 0.8
	Summer 2007	
	DOC net release	POC net release
Turf	1.46 ± 1.50	1.34 ± 0.34
Caulerpa	1.63 ± 0.81	0.48 ± 0.34
Peyssonnelia	1.57 ± 1.15	n.m.
Acropora	4.00 ± 0.70	2.24 ± 0.41
Stylophora	-3.81 ± 11.06	5.04 ± 1.77
Pocillopora	-6.75 ± 3.52	3.88 ± 0.58

Table 1: Organic matter release by the dominant benthic algae (Turf algae, green algae *Caulerpa*, red algae *Peyssonnelia*) and hermatypic corals (*Acropora*, *Stylophora*, *Pocillopora*) in the study area during the first two expeditions to the Northern Red Sea (means ± SE given as mg C m^{-2} coral or algae surface area h^{-1}; n.m = not measured; data from other expeditions not measured yet).

The comparably high DOC release by benthic reef algae in combination with the observed stimulation of planktonic microbial activity supports previously postulated statements (Kline et al. 2006, Smith et al. 2006, Dinsdale et al. 2008), which suggested that DOM released by benthic algae could stimulate microbial O_2 consumption with subsequent damage of corals in direct vicinity via hypoxia or anoxia.

Generally, algae-derived organic matter is obviously rapidly degraded in the water column, whereas this applies for coral-derived organic matter in the reef sands. Reasons for that may be that a high proportion of the algae-derived organic matter enters the DOM pool and can be taken up by planktonic microbes via the microbial loop. Kuntz et al. (2005) could demonstrate that because of this interrelationship DOM is more deleterious for corals than inorganic nutrients in reef waters.

Figure 1: Coral (upper panel) versus benthic algae dominated (lower panel) fringing reef areas in front of MSS, Aqaba, Jordan, photographed during spring expedition 2008.

Coral-derived organic matter in contrast contains more POM, which is often dominated by mucus. This material can be degraded by (specialized) microbes inhabiting the calcareous coral reef sands in high abundances (Wild et al. 2004b, Wild et al. 2005b, Wild et al. 2006), thus providing an explanation for the comparably high benthic degradation rates observed in the present study. Coral mucus in addition, because of its gel-like structure, can easily be transported via advection into the highly permeable reef sands, which act as biocatalytical particle filter systems. Such transport may not be possible to that extent for the particulate fraction of algae-derived organic matter, which can explain the pronounced advective stimulation of benthic coral-derived organic matter degradation. Algae-derived POM may in addition have a distinctive refractory character (Buchsbaum et al. 1991, Kristensen 1994), which prevents rapid degradation and leads to deposition and ultimately blockage of the reef sands. This may compromise the important function of reef sands for the recycling of organic matter and thus has potential implications for reef management.

The observed strong seasonal differences concerning algae- and coral derived organic matter release in the study area between autumn and summer were probably caused by higher availabilities of inorganic nutrients in autumn due to colder temperatures and the beginning of deep water mixing typical for the Northern Red Sea (Rasheed et al. 2002). A higher availability of inorganic matter may have resulted in increased algae growth rates and associated high synthetisation of DOM. Monitoring of benthic reef algae coverage also showed strong seasonal differences (Haas et al. unpublished data) with temporal overgrowth of reef corals by algae during late winter and early spring (see Fig. 1). However, algae blooms collapsed soon after due to depletion of inorganic nutrients in late spring. Permanent phase shifts will thus likely not appear in the study area if inorganic nutrient input from land or mariculture facilities and direct reef damage are avoided.

In summary, both investigated groups of organisms can obviously act as reef ecosystem engineers via organic matter release. However, the hard corals as “old” engineers (i.e. before phase shift) contribute differently to reef processes than benthic algae as the “new” engineers after phase shift. Element cycles via coral-derived organic matter as described by Wild et al. (2004a) contributing to the conservation of essential nutrients in the reef ecosystem will likely not take place in an algae dominated post phase shift reef, as algae-derived organic matter can apparently not substitute the important particle trapping function of coral mucus. This pilot study therefore suggests that phase shifts from coral to benthic algae may have far reaching consequences for biogeochemical processes and general reef functioning.

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Implications for Our Coral Reefs in a Changing Climate over the Next Few Decades -- Hints from the past 22 years

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Abstract. The NOAA/NASA Pathfinder sea surface temperature (SST) data from 1985 to 2006 are examined for variability and trends spanning the recent 1995-98 Pacific Decadal Oscillation (PDO) reversal to look for implications to tropical ecosystems. These twenty-two years of satellite observations reveal some noteworthy shifts that differ among major ocean basin. The data also reveal expected more rapid SST increases toward higher latitudes, especially in the northern hemisphere, consistent with Intergovernmental Panel on Climate Change (IPCC) projections. Over the 22-year timeframe, most regions showed rising trends but a few regions are presently experiencing 22-year cooling trends and thus decreasing pressure from thermal stress. What will be instructive for the future of our valuable coral reef ecosystems is whether the dramatic regional shift in trends since the recent 1995-98 PDO reversal continues to persist into the second decade of the 21st Century or if the pattern becomes overwhelmed by the pattern consistent with long-term climate change.

Key words: Climate change, SST, satellites, temperature trends.

Introduction

Our tropical oceans continue to reveal regionally diverse but changing sea surface temperature (SST) patterns and trends as we move into the 21st Century. Increasingly coral reef ecosystems have been in the news due to episodic massive coral bleaching that, in many locations, has the marine conservation community concerned about the future of their valuable habitats. In 2005, the coral community witnessed another record breaking thermal stress event throughout much of the eastern Caribbean from Puerto Rico to the British and US Virgin Islands to Barbados (Wilkinson and Souter 2008); in 2004 and 2006 Kiribati experienced massive bleaching; while in 2002 the Great Barrier Reef saw another round of extensive bleaching (http://www.reefbase.org/).

With global satellite archived SSTs now spanning more than two decades, we now have sufficient data to examine their trends not only globally but also by basin. In this paper we have taken each ocean basin in its entirety over the tropical belt and then subdivided them each into their northern and southern hemispheric component. Being aware that many climatologists have established that some of the extreme variability during the 1990s can be attributed to a mid-90s reversal in Pacific decadal patterns (Mantua et al. 1997) we have also looked at the 1st half of the 22-year record and compared it with the 2nd half.

Data

In this analysis NOAA's Coral Reef Watch (CRW) updates our climate trend statistics for the tropics using 4-km resolution Version 5.0 Pathfinder daily SST data. This builds upon our previously published analyses (Strong 1998; Strong 1991; Strong et al. 2006) by incorporating four additional years of SST data and improving the resolution of our results from 50-km to 4-km. The Pathfinder SST data are produced by retrospective analysis of daily Global Area Coverage (GAC) retrievals and are available in a variety of temporal resolutions (Casey and Cornillon 1999; Kilpatrick et al. 2001). Data are available at http://www.nodc.noaa.gov/SatelliteData/pathfinder4km/.

We analyzed global satellite-derived SSTs from 35ºN to 35ºS from 1985 to 2006. Continued month-by-month comparisons of coincident *in situ* and SSTs at various locations around the global oceans reveal tight correlations and, most importantly, no biases between the buoy-measured and remotely-sensed data sets over time (not shown). Therefore we have the ability over this time period to examine our tropical oceans more completely than had been possible with *in situ* SST measurements, baring prolonged periods of cloudiness where SSTs become obscured from the view of the satellite – fortunately this is more of an issue at higher latitudes than for the tropics. Since the satellite-derived Pathfinder SSTs come from

continuous, overlapping, operational NOAA satellites, they have what we believe to be the best possible climate-quality global SST data since 1985.

In calculating SST trends for the 22-year time frame from the daily 4-km Pathfinder SSTs, we used only the daily nighttime SSTs. This was done to avoid the error caused by uneven day-night data distribution for places that experience significant diel variation in SST caused by daytime solar heating and nighttime cooling, especially during calm weather conditions. First, only high quality SST values were used (quality flag ≥ 4 as set in the Pathfinder processing — in a scale ranging from 0 to 7, lowest to highest quality) (Kilpatrick et al. 2001). The monthly mean SST at each 4-km pixel was calculated by averaging all the available, high quality daily nighttime SSTs in the 3x3 pixel box centered on the pixel where the SST is made. If a pixel contained no qualified data during a month, a missing data flag was assigned to that pixel for that particular month. Based on the resulting monthly mean SSTs, for every pixel, one round of temporal linear interpolation was conducted to fill missing monthly mean SSTs using the monthly mean SSTs of the prior and subsequent month if both are available. No further missing data filling was performed for monthly mean SSTs. Finally, our annual mean SSTs at each pixel were calculated from the 12 monthly mean SSTs at the pixel, where we required that all 12 monthly mean SSTs had to be available for an acceptable calculation of an annual mean for that year; otherwise we flagged the value as missing data for that year. SST trends were calculated from the annual mean SSTs for all pixels that had annual mean SSTs available for at least half of the total number of the years covered.

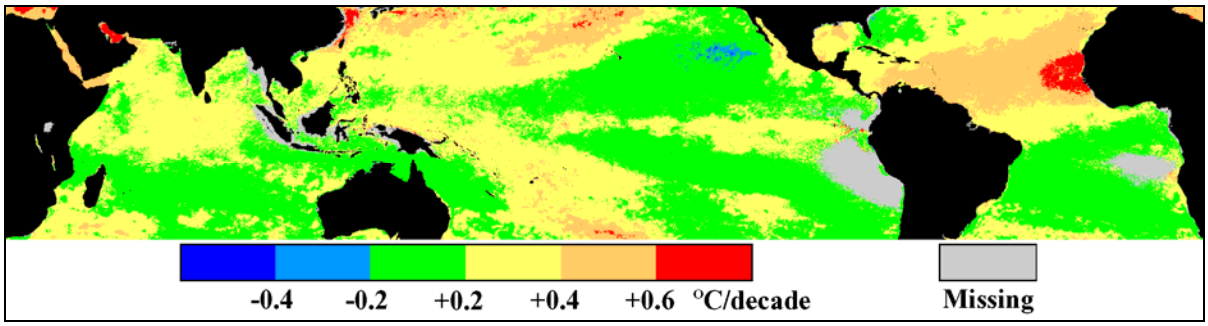

Figure 1: 22-year satellite SST Trends (1985-2006)

Global Images of Changing SSTs

In Fig. 1 we present our SST trends from monthly mean SSTs at 4-km resolution for the 22-year time frame. The pixels where the availability of SST data is not sufficient to calculate a trend, as explained in the preceding section, show up as areas of "gray" in the Fig. 1 and principally occupy areas off western S. America, west of Baja California, the region close to and surrounding Indonesia and Papua New Guinea, as well as the south Atlantic off Africa. These areas do, in fact, have persistent cloud cover that has resulted in a few months of data being hidden from the view of the NOAA satellite radiometers. Areas shown in green demonstrated weak cooling or warming (-0.2 to +0.2°C /decade) for the period while those areas depicted as more orange or red demonstrated much higher increases in SST. In the 22-year period, the entire tropical region demonstrated either warming or very slight cooling (> -2°C/decade) – except for the small "blue" (≤ -2°C/decade) region off Mexico in the northeastern tropical Pacific Ocean.

When we examine the tendencies over two 11-year intervals that straddle the mid-1990s shift in Pacific decadal temperatures, often referred to as the pacific Decadal Oscillation or PDO (Mantua et al. 1997; Bondi et al. 2001), we observe much more local variability and some fairly robust 11-year trends (Fig. 2). Whereas the earlier 11-year interval (1985-1995) shows some downward SST trends in the tropics, all of those regions (predominantly in the southern hemisphere) exhibited pronounced reversals in the following 11 years (1996-2006).

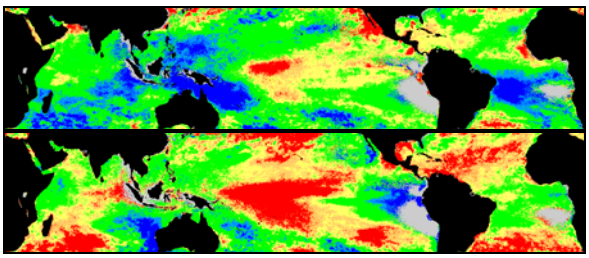
Figure 2: SST tendencies for first 11 years [top: 1985-1995] and second 11 years [bottom: 1985-1995]. Color scale as for Fig. 1.

Fig. 3 provides 22-year trends calculated from mean annual SST, both globally and divided by

hemisphere. Over 95% of the tropical ocean pixels gave cloud-free SSTs that went into these calculations. Mean tropical SSTs increased at a rate of + 0.21 °C/decade with a northern tropical ocean increase one and a half times as fast as that of the southern tropics.

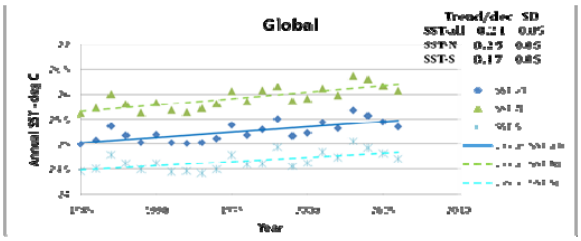

Figure 3: Tropical [35N-35S] yearly SST means with trends; global tropics and by hemisphere.

Fig. 4 plots the zonal oceanic zonal means of the 1985-2006 SST trends at 4-km resolution between 35S and 35N. For each line of latitude, the mean value is calculated from all data available at that latitudinal row.

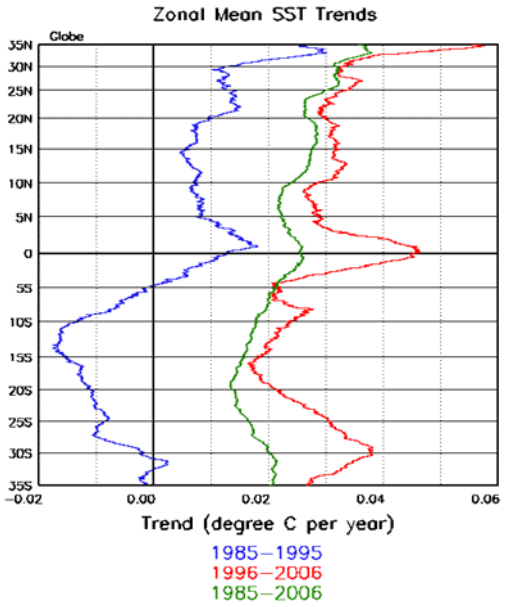

Figure 4: Trends averaged across latitudinal pixels: 1985-1996[blue], 1996-2006[red], and 1985-2006[green]

This mean row value is considered as the mean value for all of the water pixels in that row. For most rows, the percentage of missing data pixels is very low (< 5%). The y-axis, i.e., latitude axis, is not linear and is plotted in proportion to the variation of the water surface area in the meridional direction as the result of both the change in latitude and change in landmass at different latitudes.

Some Representative Virtual Station Trends

In our collaborations with marine park managers, CRW maintains a set of 24 representative operational Virtual Stations around the globe (Liu et al. 2006) spanning all ocean basins (in late-2008 an additional 166 stations were added as "experimental" Virtual Stations – http://coralreefwatch.noaa.gov). Operational stations are maintained "24/7" and the data for these stations continue from satellite to satellite as the NOAA observing system is maintained seamlessly through time, with routine verifications/calibrations preserving data quality and continuity. The new 166 sites are termed "experimental" Virtual Stations as the representativeness and final location of these sites is under evaluation. Below, the trends at some of the operational and experimental Virtual Station sites are discussed, along with some other representative sites selected that help extend this trend analysis across the tropics. Fig. 5 provides trends at these selected sites.

Caribbean – Atlantic Ocean

Four selected operational northern hemisphere, Caribbean-Atlantic Virtual Station sites are shown here (Fig. 5a). SST trends range from a low of +0.22°C/dec at Puerto Morelos and Lee Stocking Island (Bahamas) to a high nearly twice that at the US Virgin Islands.

Puerto Morelos:	+ **0.22°C/dec** [**σ** = 0.06]
USVI:	+ **0.41°C/dec** [**σ** = 0.10]
Bahamas [Lee Stocking]:	+ **0.22°C/dec** [**σ** = 0.09]
Bermuda:	+ **0.27°C/dec** [**σ** = 0.08]

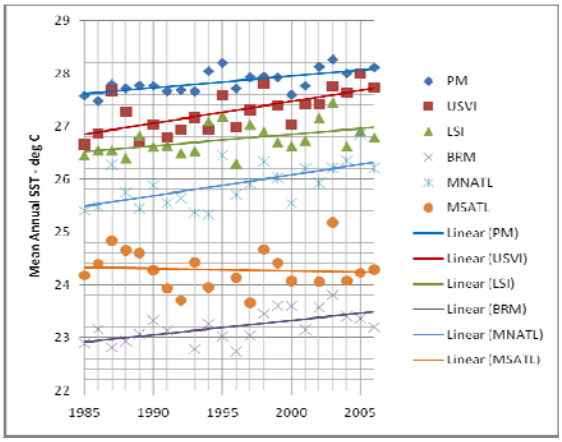

Figure 5a: Atlantic Ocean Virtual Stations

Mid-Atlantic Ocean - Extremes

Two additional middle Atlantic SST Virtual Stations were considered as mid-ocean examples on either side of the equator (Fig. 5a). Trends [as can be seen Fig. 1] are high in the North Atlantic but there is no significant trend in the South Atlantic.

Mid N. Atlantic: + **0.39°C/dec** [**σ** = 0.11]
Mid S. Atlantic: - **0.04°C/dec** [**σ** = 0.13]

Indian Ocean

The three Virtual Stations across the Indian Ocean (Fig. 5c) show the most dramatic increase in SSTs in the Seychelles, +0.30°C/dec, and lower rise of +0.19°C/dec at our Zanzibar site.

Zanzibar: + **0.19°C/dec** [**σ** = 0.08]
Seychelles: + **0.30°C/dec** [**σ** = 0.12]
Scott Reef: + **0.24°C/dec** [**σ** = 0.14]

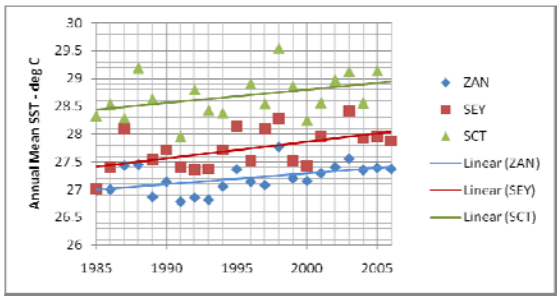

Figure 5c: Indian Ocean Virtual Stations.

Pacific Ocean

We found quite a range of increasing SST trends over the Pacific as represented by seven example Virtual Stations (Fig. 5b) from +0.38°C/dec at Bolinao, Philippines to only +0.19°C/dec at Heron Island, Australia.

Midway: + **0.34°C/dec** [**σ** = 0.17]
Guam: + **0.36°C/dec** [**σ** = 0.15]
Kiribati: + **0.20°C/dec** [**σ** = 0.25]
Palau: + **0.29°C/dec** [**σ** = 0.18]
Bolinao: + **0.38°C/dec** [**σ** = 0.17]
Heron Island: + **0.19°C/dec** [**σ** = 0.11]
Oahu: + **0.20°C/dec** [**σ** = 0.12]

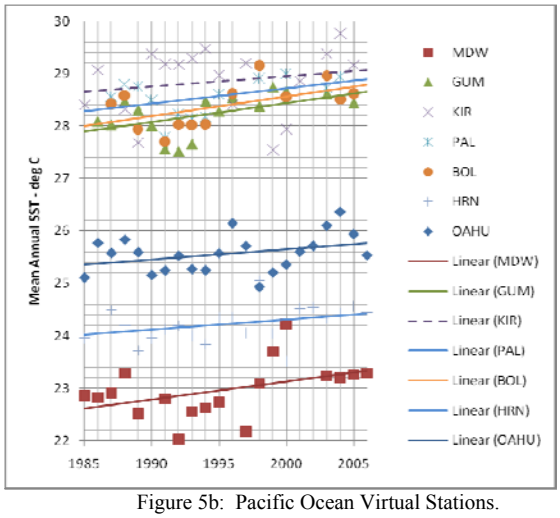

Figure 5b: Pacific Ocean Virtual Stations.

These analyses at our Virtual Stations suggest significant variability between and within oceanic basins.

Regional Trends

Examining our data by ocean basin shows interesting patterns. The greatest rate of warming has occurred in the North Atlantic. The Southern basins continue to lag behind with trends nearly half those found north of the Equator – the South Pacific rate is nearly one third that of the tropical North Atlantic, offset primarily by the weaker rises over much of the eastern portion of each region.

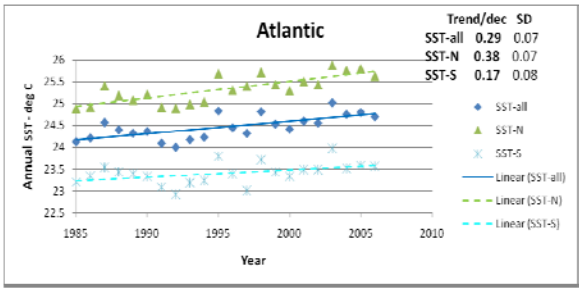

Figure 6a: Atlantic Ocean Trends.

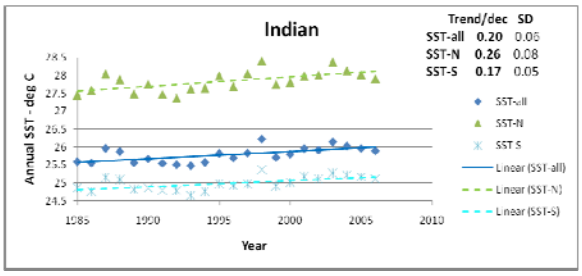

Figure 6b: Indian Ocean Trends.

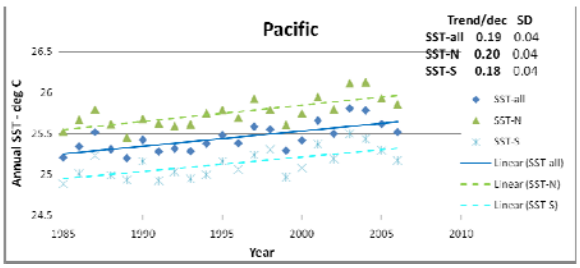

Figure 6c: Pacific Ocean Trends.

Discussion

We have presented a case for increasing ocean SSTs, first with only 8 years (1982-1989) of SST satellite data (Strong 1998; Strong 1991), then 10 years later (Strong et al. 2006), and now with 22 years of rigorously reconstructed Pathfinder SSTs. Under the growing concern for continued rising temperatures looming for the rest of this century, we can see some fairly consistent trends stabilizing with a longer

record over the global tropics. Although most of our coral reef sites tend to favor the warmer non-upwelling sides of each ocean basin (where corals grow best) and are confined within 35 degrees of latitude on either side of the Equator, they are often in regions that appear to have some of the higher warming trends in their respective basins. This would be expected since the eastern sides of each basin are more influenced by upwelling (and increased cloud cover) that introduces cooler water from below helping to stabilize our warming oceans. With convergent water movement and a tendency more toward sinking water columns in the tropics on the western sides of these basins, warmer surface waters accumulate heat in the upper layer more quickly, where these reefs thrive, thus favoring SST trends that would be expected to be larger than those of their eastern region (upwelling) counterparts.

Since the overall calculations of our tropical SST trends have missed several percent of the total sea surface area where cooler SSTs persist due to frequent persistent cloud cover, we might expect the trends for our total areas (all pixels) in each basin to be slightly less than what has been shown if we were able to incorporate these SST pixels (cloudy/upwelling) into our calculations (this will eventually become possible when satellite microwave SSTs become integrated into our observation program of SSTs).

We were able to process several years of archived operational SST annual mean SSTs (http://coralreefwatch.noaa.gov) for our Virtual Station sites to compare with the Pathfinder SSTs. These results (not shown) revealed nothing unusual and were virtually coincident giving us confidence in the accuracy of both our operational SSTs and re-processed Pathfinder SSTs. These results all consistently point out that coral reefs are already experiencing rapid temperatures increases beyond those to which they may be able to adapt (Donner et al. 2005; Hoegh-Guldberg et al. 2007).

An interesting finding that can be seen in each of the three tropical ocean basins is that 2003 has been our warmest year of the 22-year record. Since that time SSTs have been gradually dropping, indicating a downward shift from the long-term trend. If this is a result due to the recent PDO reversal there might be some hope for our thermally stressed ecosystems. 17 of our 24 Virtual Stations showed cooler than normal SSTs during the first half of 2008, including some of the lowest monthly values seen this century! If this reduced short-term trend continues to prevail there is a glimmer of hope for many of our coral reefs for the next decade or two. The long term concern is the path of temperatures after the next PDO reversal, perhaps in the mid-2010s, when SST increases might surpass those seen during the 80s and 90s reaching yet higher levels of thermal stress (Hoegh-Guldberg et al. 2008). Based on our results and continued trends, unless coral reefs are able to develop coping mechanisms for increased thermal stress during the next decade, the flowing decades will only continue to put our fragile reefs under increased concern.

Acknowledgement

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Adaptation/Exaptation in the host coral *Favites complanata* (Ehrenberg, 1834; Scleractinia, Faviidae) to increased Seawater Temperatures

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Abstract. Recent evidence suggests that, as global climate changes and seawater temperatures rise, the primary cause of coral bleaching is zooxantheallar mortality indicated by cell apoptosis/necrosis. But are the coral hosts themselves sensitive to increasing temperatures, and, if so, to what degree? We exposed the coral *Favites complanata* (Ehrenberg, 1834) with their symbiotic zooxanthellae (*Symbiodinium* sp.) to experimental temperatures of 28 (control), 30, 32, and 34°C for 12h. We assessed coral and symbiont cells *in situ* for symptoms of apoptosis and necrosis using transmission electron microscopy (TEM). *F. complanata* host cells *in situ* exhibited little or no mortality from exposure to increased seawater temperatures, except at very high temperatures (34°C) for ≥12hrs. By contrast, we found high levels of apoptosis/necrosis in the zooxanthellae *in situ* under all experimentally elevated temperatures. These findings indicate that the host corals are adapted/exapted to seawater temperature increases. They also imply that the coral hosts do not suffer mortality from temperature stress but from loss of their symbionts – a process driving natural selection in *Symbiodinium* and most likely resulting in their rapid adaptation to a changing environment.

Key words: Adaptation, Apoptosis, Bleaching, Exaptation, Necrosis, Seawater temperature, *Symbiodinium*

Introduction

Concerns are growing as climate change and associated increased seawater temperatures continue to induce coral bleaching in the tropics and sub-tropics (Sheppard and Rioja-Jieto 2005; Baker et al. 2008). Coral bleaching, a process by which corals lose their algal symbionts called zooxanthellae (genus *Symbiodinium*), is a response to heat-stress where temperatures rise above the maximum tolerance of the zooxanthellate corals/holobionts (Hoegh-Guldberg 1999). Such variation in temperature may be an important selective factor in the evolution of the relationship between corals and their endosymbionts. Some investigators suggest that the loss of *Symbiodinium* cells may help corals adapt to changing environmental conditions by allowing them to "shuffle" (Buddemeier and Fautin 1993) their complement of zooxanthellae, benefiting the corals (the "adaptive bleaching hypothesis"; also see Kinzie et al. 2001). Others believe that *Symbiodinium* shuffling does not occur quickly enough (Hoegh-Guldberg et al. 2002), serving to prolong the eventual death of the host. The interim survivorship period may last for up to six weeks. Grottoli et al. (2006) have demonstrated that *Montipora capitata* can replenish its energy reserves and biomass in the absence of its symbionts by increasing its food intake five-fold after temperature-induced bleaching. Borell et al. (2008) found similar effects in *Stylophora pistillata* (Esper) and *Galaxea fascicularis* (Linnaeus).

Recent data have indicated that bleaching is a primary selective factor for zooxanthellae, causing very high levels of mortality in zooxanthellae *ex situ* (Strychar et al. 2004 a, b; 2005). This can be inferred from the 95-99% mortality rate of zooxanthellae expelled from scleractinian and alcyonacean corals exposed to temperatures of 32-34°C for 9-48 h (*ibid.*).

Survival of zooxanthellae exposed to high seawater temperatures may occur as a result of adaptation by ancestors that have survived prior exposure to these conditions. Survival may also be occurring due to exaptation to these temperatures. Exaptation is defined as a character that has evolved for another function, or no function at all, but which has been co-opted for a new use (Gould and Vrba 1982, cf Futuyma 1998; McLennan 2008; also see Strychar and Sammarco, 2009). Mettler and Gregg (1969) discuss exaptation, previously termed "pre-adaptation", as follows: "…populations can… generate many genotypes with varying degrees of adaptedness. Those [non-adapted] forms produced each generation…are weeded out by selection, but …are continuously produced and …considered to be 'stores on hand'…the progenitors of future generations in the event of a changed environment. They are 'pre-adapted' genotypes ready for new

situations which might be met by the population…."

The high mortality rates observed by Strychar et al. (2004a,b, 2005) were determined *via* assessing apoptosis and necrosis in the symbiont cells. Apoptosis is a genetically pre-programmed, physiological and biochemical event that leads to the removal of unwanted and/or abnormal cells (Strychar et al., 2004a). This helps to regulate abundant cell populations. Apoptosis is not a synonym for programmed cell death, which involve other mechanisms (e.g. paraptosis, pyroptosis, etc.; see reviews of Ameisen 2002; Fink and Cookson 2005). Necrosis, by contrast, is a passive, accidental, and unordered occurrence of cellular death (Strychar et al., 2004a), causing inflammation of host tissue. For example, increased production of apoptotic cells in mammalian hosts elicits removal of dead/dying cells *via* phagocytosis, preventing inflammation. Necrosis, on the other hand, causes uncontrolled inflammation (Fink and Cookson, 2005). In Cnidarians, increased concentrations of necrotic cells likely cause similar responses. In zooxanthellate corals, the occurrence of apoptosis or necrosis in the symbiont but not the host would indicate that the former is more sensitive to a perturbation than the latter.

The rate of coral mortality due to bleaching is believed dependent upon the severity (intensity and duration) of the temperature anomaly (Winter et al. 1998, 2006; Craig et al. 2001; but also see Podesta and Glynn 1997). We have shown that some host corals, specifically *Acropora hyacinthus* (Dana, 1846; Acroporidae) and *Porites solida* (Forskål, 1775; Poritidae) from the Great Barrier Reef, Australia have a much broader temperature tolerance than that of the zooxanthellae and exhibit adaptation/exaptation towards increased temperatures (Strychar and Sammarco, 2009). The zooxanthellae appear to be doing most of the adaptation within this co-evolved symbiotic relationship. Here we demonstrate that this phenomenon is not restricted to these two species, or families, but may be extended to *Favites complanata* (Ehrenberg, 1834; Faviidae), and that this adaptation / exaptation may be a common characteristic within the Scleractinia.

Material and Methods

Forty colonies of *Favites complanata* were collected at ~8.5m depth from Barren Reef (23°10'S, 151°55'E), Great Barrier Reef, Qld, Australia. Colonies were exposed to different experimental seawater temperatures, following a random blocks design (Zar 1998). Full details of the experimental design may be found in Strychar et al. (2004b) but will be summarized here. Filtered seawater was pumped through 10 and 1µm filters and deposited into a series of header tanks (total combined vol = 1000 l), mixed using submersible pumps. Water was then pumped to 8 two-liter plastic holding chambers at a constant flow rate of 20ml min^{-1} through 1µm Millipore® filters. Magnetic stir bars provided water movement within each chamber. Colonies were subjected to experimental temperatures of 28 (control), 30, 32, and 34°C for 12h. Ten replicate (n_i=10) coral fragments were used for each temperature. Data presented here are derived from experiments described in Strychar et al. (2004b); here, however, the focus has shifted to host and *Symbiodinium* cells *in situ*, as opposed to expelled cells. We acknowledge that the rates of temperature increase and/or time scales of exposure employed here are faster and/or shorter than may occur naturally in the reef environment. Hence, the coral and symbiont experimental responses may not be representative of a protracted natural bleaching event.

In these experiments, coral tissue (~5mm^2) was excised from a colony using a scalpel and fine dissecting forceps at the 3, 9, & 12h marks. A solution of sodium citrate (10%) and formic acid (20%) was used to decalcify skeletal tissue (Strychar and Sammarco, 2009). Any skeletal tissue remaining after 24h was gently crushed using a micro-tissue grinder, and the solution was centrifuged and washed 3x in phosphate buffered saline solution (PBS). Following a 3rd wash and removal of the supernatant, samples of host tissue, along with the symbiont cells, were embedded in 0.2ml of agar. Thus, both the symbiotic partners could be analyzed simultaneously. After solidification, samples were post-fixed in 1% osmium tetroxide in 0.1 M PBS for 24h at 48°C. They were then centrifuged (500x *g*) and the supernatant removed. At 30 min intervals, the samples were dehydrated in a graded series of acetone washes (30, 50, 70, 90, & 3x at 100%). The embedded samples were then suspended in a series of graded acetone–Spurr's resin to ensure infiltration of resin into the tissue. Samples were finally polymerized into a mould by incubation at 60°C for 3d.

An Ultracut T microtome was used to prepare thin sections of tissue, which were placed on 3mm copper grids coated with 1.2% (w/v) formvar in trichloro-methane. Aqueous uranyl acetate was then used to stain the thin sections for ~5 min, followed by staining with 1.5% (w/v) lead citrate for 5 min. The ultrastructural characteristics of each thin section were observed with a JEOL-1010 or Hitachi 7000 transmission electron microscope (TEM) and analyzed for ultrastructural symptoms of apoptosis, necrosis, and viability. Thin sections were examined during each sampling period; 100 host and 100 symbiont cells were randomly sampled per section per experimental temperature treatment at each time interval. Frequencies of each were calculated.

Cells exhibiting apoptosis were characterized by cell contraction, chromatin condensation, and cell fragmentation into membrane-bound fragments called apoptotic bodies (see Strychar et al., 2004a). Necrotic cells were identified by cellular and organelle swelling, eventually causing release of the cytosolic contents into the surrounding host tissue.

Results

Coral host and* Symbiodinium *cells – Main Effects

TEM successfully detected variation in host and *Symbiodinium* cell states under varying seawater temperatures. There was no significant difference in apoptosis and necrosis levels between the 28°C and 30°C treatments in either the *Favites complanata* host cells or the *Symbiodinium* cells ($p>0.05$, MANOVA). Apoptosis levels increased significantly, however, in host corals exposed to temperatures ≥32°C (main effects; $p<0.05$, MANOVA). Levels of necrosis in host cells did not change significantly under conditions of increased temperatures *in situ* ($p>0.05$). Endosymbiont cells exhibited significantly higher levels of apoptosis at temperatures >30°C ($p<0.05$). Necrosis was significantly more frequent in the symbionts at temperature treatments >30°C *vs.* 28°C (control). Symptoms of stress in host corals and their symbionts (i.e. apoptosis & necrosis) rose significantly >9h after initiation of the experiment (main effect – time; $p<0.05$, MANOVA).

Temporal Responses of* Favites complanata *host cells and symbiont cells* in-situ *at 28-34°C. At 28°C, both host and symbiont cells exhibited high percentages of viability *in situ* (Fig. 1). The number of viable symbiont cells, however, decreased markedly and significantly as experimental temperatures were increased from 28 to 34°C ($p<0.001$, Tukey HSD and Bonferroni; Fig. 1).

Fig. 2 illustrates how the frequencies of apoptotic cells in both host and symbionts were negligible at experimental treatments of 28-30°C through time. At 32°C and 34°C, however, the level of apoptosis increased significantly through time in the *Symbiodinium* cells ($p<0.001$, Tukey HSD and Bonferroni tests). The frequency of apoptosis in *Favia complanata* host cells *in situ* rose significantly only under the extreme experimental temperature of 34°C, when compared to the control ($p<0.05$).

The frequency of necrosis in the host cells *in situ* did not vary significantly between any of the experimental temperatures ($p>0.05$, Tukey HSD and Bonferroni tests; Fig. 3). By contrast, *Symbiodinium* cells exhibited significantly higher frequencies of necrosis at 34°C ($p<0.04$; Fig. 3).

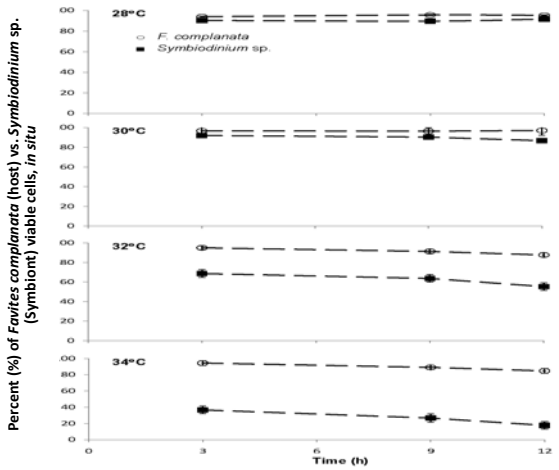

Figure 1: Effect of temperature on the relative frequencies of **viable** *Favites complanata* host and *Symbiodinium* cells *in-situ,* collected over 12h. Data are presented as the mean plus 95% confidence intervals from nine separate experiments; some confidence limits, too small to be seen. Each point = percentage of different cell types. The frequency of viable cells in *Symbiodinium* at 32°C and 34°C treatments were each significantly lower than all other treatments ($p<0.05$, Tukey HSD test). There was no significant difference between the 30°C and 32°C treatments in the host coral ($p>0.05$), no significant difference between the 28°C and 30°C treatments, and the 34°C treatment was significantly lower than the other treatments ($p<0.05$). There was an significant decrease in frequency of viable cells in both *Symbiodinium* and in the host cells between 3 and 12 hrs ($p<0.001$ & $p<0.05$, respectively).

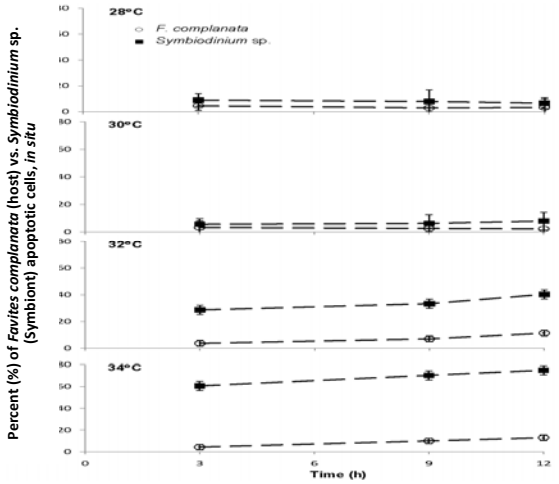

Figure 2: Effect of temperature on the relative frequencies of **apoptotic** *Favites complanata* host cells and *Symbiodinium* cells *in-situ,* collected over 12h. Data presented as the mean plus 95% confidence intervals; some confidence limits, too small to be seen. Each point = percentage of different cell types. The apoptotic cell frequencies in *Symbiodinium* at 32°C and 34°C were each significantly higher than all other treatments ($p<0.05$, Tukey HSD test). There was no significant difference between the 28°C and 30°C treatments ($p>0.05$). Apoptotic cell frequencies in the host cells were significantly higher at 34°C than at 28°C and 30°C ($p<0.05$). There was no significant difference between the 28°C and 30°C treatments ($p>0.05$). There was a significant increase in apoptotic *Symbiodinium* cells between 3 and 12hrs ($p<0.05$), but no difference in the host cells during this period ($p>0.05$).

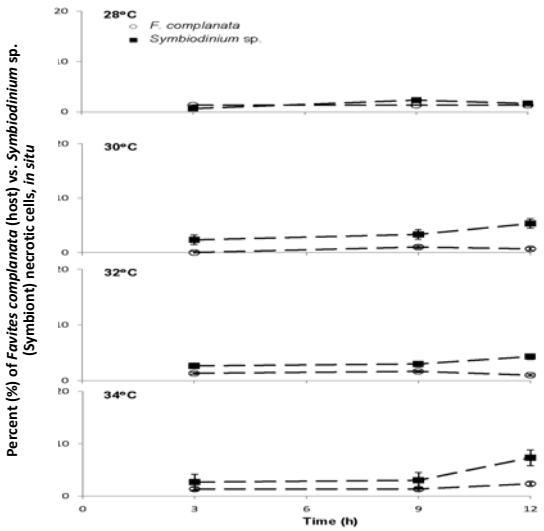

Figure 3: Effect of temperature on the percentage of **necrotic** *Favites complanata* host cells and *Symbiodinium* cells *in-situ,* collected over 12h. Data are presented as the mean plus 95% confidence intervals; some confidence limits, too small to be seen. Each point = percentage of different cell types. The frequency of necrotic cells in *Symbiodinium* at 34ºC was significantly higher than at 28ºC ($p<0.05$, Tukey HSD test). There was no significant difference in necrotic host cell frequencies between any temperature treatments ($p>0.05$). There was a significant increase in necrotic *Symbiodinium* cells between 3 and 12hrs ($p<0.05$), but no significant change in necrosis in host cells during that period ($p>0.05$).

Discussion

Our data indicate that the scleractinian coral *Favites complanata* is less heat-sensitive than its endo-symbionts. We hypothesize that increasing seawater temperatures, characteristic of global warming associated with climate change may be acting as a selective factor on the symbiotic zooxanthellae within the coral host, but not on the coral hosts themselves. This is because the coral hosts are already either adapted/exapted to high seawater temperatures. We have already desmonstrated adaptation/exaptation in the host corals *Acropora hyacinthus* and *Porites solida* - families Acroporidae and Poritidae, respectively. The data here show that this characteristic is restricted not only to these two species or families – but may also be extended to *Favites complanata* and its family – the Faviidae, suggesting that such temperature tolerances may be a widely distributed character in Scleractinain corals.

Like earlier studies, this suggests that it is the zooxanthellae that are responsible for doing most of the dying in this symbiotic relationship and the lion's share of the adaptation to rising seawater temperatures, certainly in the central Great Barrier Reef region. We propose that mortality is much more frequent and severe in the zooxanthellar populations during bleaching, and that the corals are suffering not from heat stress but from loss of their symbiotic partners. This is consistent with the results of Mise and Hidaka (2003) who studied effects of increased temperature on *Acropora nasuta*. They described rapid degradation of zooxanthellar cells under similar conditions, detailing symptoms characteristic of apoptosis and necrosis (also see Strychar et al., 2004a), and suggested that "degraded zooxanthellae are produced by host digestion". This implies that the host controls the disposal of the compromised portion of the zooxanthellar population *in situ*. Based on our data, we believe this may be the case.

Whether seawater temperatures rose above 32°C in the evolutionary past of the host animals, or whether the genes responsible for this tolerance originally applied to some other function, is not known. Their current function, however, is clear. *Symbiodinium* appears to still be adapting to current increases in SSTs, at its own rate. Can adaptation occur in both the host and the symbiont? We believe so. The literature suggests that it is quite possible for the algal symbionts to become adapted to these changes. For example, corals and their symbionts are known to thrive in environments in the Red Sea where SSTs routinely reach 34°C (Ateweberhan et al., 2005) and salinities reach up to 40.5ppt (Karako-Lampert et al., 2004).

If our higher temperatures were approached at a slower rate, this could have allowed time for some symbiont shuffling, lessening symptoms of bleaching. This could have decreased levels apoptosis/necrosis in the symbionts when higher temperatures were reached. The effects of slow temperature increases on symbiont shuffling, however, remains to be demonstrated, in the field or lab.

Here we have considered only *in situ* cells. Earlier (Strychar et al., 2004b), we demonstrated that *Symbiodinium* cell fractions expelled from the host exhibited apoptotic or necrotic characteristics with increased seawater temperature and time (up to 48h).This further supports the concept that the host cells are better adapted to elevated temperatures than their symbiotic zooxanthellae. We have also reported that we could only identify one clade of symbiont in *Favites complanata* - clade C (Strychar et al., 2005). Not all *F. complanata* have the same clade of symbionts, however. *Symbiodinium* clades A, B, C, D (Baker and Romanski 2007), and C3 (LaJeunesse et al., 2003) have all been identified from members of the Faviidae. It is possible that *F. complanata* could associate with multiple symbiont clades; thus, the response observed in our experimental colonies may not be representative of all *F. complanata* holobionts.

The adaptation/exaptation of a host coral to elevated seawater temperatures has now been demonstrated in three different hermatypic corals

from three separate families – the Faviidae, Acroporidae, and Poritidae – from the Great Barrier Reef. These corals and possibly families of corals are either already adapted to tolerate high temperatures from their ancestors having survived past high-temperature events in their evolutionary history, or they are exapted (Gould and Vrba, 1982; cf Futuyma, 1998; McLennan 2008) to these high temperatures *via* a pre-existing character associated with another physiological function. In the case of exaptation, it is not possible to discern from what character this tolerance may have been derived.

We thus hypothesize that this character is widespread among the Scleractinia of the Great Barrier Reef, and that death of the coral host in these organisms is due to their dependency upon the nutrients or other benefits that the symbionts provide. Coral hosts appear to be well adapted to temperature stress, while their endosymbiotic zooxanthellae either die or experience irreversible cell damage while still embedded in the coral host tissue, prior to expulsion from the coral. If the symbionts are not replaced or the loss of nutrients is not supplemented within some given time period, the host may die.

Adaptation and physiological tolerance to higher than normal temperatures while maintaining normal cell and tissue function is the key to survival in both the host corals and their symbiotic zooxanthellae. It would appear that it is the inherited adaptation or exaptation of pre-existing physiological traits from some ancestor that will permit survival of corals through its application to an environmental stress, the severity of which is increasing with time.

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GIS modeling and mitigation of coral reef damage

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Abstract. The objective of this study is to demonstrate that an efficient instrument for conducting surveys and inventories of coral reefs to assess those ecosystems at higher risk and develop mitigation strategies is through the use of a Geographic Information System (GIS). Efficient monitoring requires the assessment of various coastal data baselines and the evaluation of subsequent alterations in spatial patterns. While monitoring involves real-time components, among the most powerful tools of a GIS are its modeling capabilities, which allow simulation of various climate change scenarios. Relevant aspects include changes in coastal land use, wetlands, and shoreline configuration. Using Caribbean coral reef examples, the results of this research reveal that GIS techniques and applications play an integral role in defending coral reefs from climate change and other threats. Planners and politicians require the ability to analyze risks, assess impacts, and consider alternatives based on input from researchers across diverse disciplines. GIS provides the collective tool that integrates multifaceted data and transforms it into a meaningful medium for informed decision-making.

Key words: Geographic Information Systems, reef monitoring, reef management

Introduction

According to the most recent assessments from the Intergovernmental Panel on Climate Change (Mann & Kump 2009), as a result of climate change and sea level rise, the planet's coasts are exposed to multiple risks including coastal erosion, changes in sediment and nutrient transport, and additional coastal flooding. Heavily populated, low-lying areas, which already face other challenges such as tropical storms or local coastal subsidence, are especially vulnerable. Coastal wetlands, including mangroves and salt marshes, will be impacted by sea-level rise especially in places where they are inhibited from migration as a result of natural or anthropogenic barriers. Despite their diversity, coral reefs are highly susceptible to thermal stress and have low adaptive capacity. Increases in sea surface temperature of 1 to 3 degrees Celsius are projected to result in more frequent coral bleaching events and widespread mortality with increasing human-induced pressures on coastal areas aggravating these negative effects. A Geographic Information System (GIS) is a tool that can create, analyze, and display sea level rise scenarios helping managers address the negative effects of elevated sea levels by allowing them to identify coral reef communities that are at risk, assess the situation, and develop mitigation strategies.

Coastal Ecology and Economy

According to Cohen et al. (1997), 20% of the people in the world live within 30 kilometers of the coast and it is predicted that by the year 2100, approximately 600 million humans will live on coastal floodplains lower than the 1000-year flood stage (Nicholls & Mimura 1998). Burns (2000) reached similar conclusions regarding island populations reporting that most island inhabitants, along with their infrastructure and socioeconomic activities, are situated just a few hundred meters from the shore and as a result, are likely to experience negative impacts from rising sea-levels. The destructive effects could include coastal flooding, loss of wetlands, saltwater intrusion, increased erosion, and higher storm surges. Additionally, these impacts have the potential to affect many of the smaller islands in the Caribbean due to certain similar traits these islands share such as limited funds, natural resources, geographic size, and infrastructure (Leatherman 1997).

Granger (1997) states that the projected sea level rise could seriously damage the socioeconomic growth of smaller island states while Leatherman (1997) asserts that practically every social and economic sector will be disrupted. Smaller low islands might not have the physical size to deal with rising sea level, and residents might be forced to relocate to other countries, which could have dire socioeconomic costs (Nicholls & Mimura 1998). For example, the IPCC (2001) projects that on the island of Cuba a 1-meter rise in sea level would displace 50,000 people. Nicholls et al. (1999) suggest that 22% of coastal wetlands could be lost as a result of rising sea levels by the year 2080 and that the Caribbean Islands along with those of the Pacific and Indian Oceans will experience the greatest relative

rise in frequent flooding. Over the past 15 years, some beaches on Trinidad and Tobago have reported annual erosion rates of 2-4 meters, which is primarily attributed to rising sea levels (Singh 1997).

Flooding and erosion also could adversely affect the tourism industry, which is a major economic sector on many islands of the Caribbean through the loss of beaches, saltwater intrusion, infrastructure damage, and other forms of degradation. Most of the islands with economies based primarily on tourism tend to concentrate their resources and infrastructure along the coasts where rising sea levels will have the greatest impact. The problem will be compounded by the fact that many existing tourist facilities currently are not adequate and frequently experience problems such as water shortages.

Many of the Caribbean Islands meet their water demands through an exclusive source such as rainwater, groundwater, importing, or surface reservoirs, which makes them vulnerable to a disruption in supply. For those islands that rely on precipitation stored during the rainy season, any variation in climatic patterns could be critical. The IPCC (2001) points out that on Barbados three months of the rainy season account for all of the groundwater recharge and, just 15% to 30% of the yearly precipitation actually makes it to the aquifer.

However, perhaps the most critical concern is the threat of saltwater intrusion, which is occurring on some islands, such as Barbados, due to over pumping. With an increase in sea level, intrusion could take place at pumping stations further inland as well as along the coasts. On Trinidad and Tobago, over pumping, which has been aggravated by rising sea levels, has contributed to a significant salinity increase among some coastal aquifers (Singh 1997).

Mangrove forests are an integral component of the island ecosystem offering protection from high tides and storm surges, while providing feeding grounds for numerous species juvenile fish and products. However, many mangrove habitats in the Caribbean are suffering from human exploitation and will face further stress with rising sea levels. According to the IPCC (2001), 62% of the mangroves in Puerto Rico have been eradicated due to anthropogenic activity, and a 1-meter sea level rise in Cuba could destroy 30% of that island's mangrove ecosystems. Alleng (1998) reports that due to the inability to migrate, Jamaica's Port Royal mangrove forest could be totally devastated with rising sea levels. Consequently, the loss of mangrove forests will have negative effects on local wildlife. For example, Sattersfield et al. (1998) observe that the forested areas of the Caribbean are home to most of the threatened bird species in the region.

Likewise, coral reefs supply food and shelter for marine flora and fauna while providing a natural barrier as well as income in the form of tourism and fishing for the Caribbean islands. However, a global inventory of Earth's coral reefs estimates that already nearly 60% are at risk due to the actions of humans (Bryant et al. 1997), and 90% of all living reefs have been damaged by coral bleaching (Goreau & Hayes, 1994). Regionally, the occurrence of coral bleaching will be the highest in the Caribbean and could take place on an annual basis over the course of the next 30-50 years (Hoegh-Guldberg 1999).

GIS Modeling and Mitigation

Among the steps that can be taken toward informing the public of these threats is the modeling of various sea-level scenarios using GIS. Beginning with the establishment of mean sea level for a particular location based on topographic quadrangle contour lines, which are the most valuable data for determining which locations might be inundated, it is possible to adjust sea-levels according to estimated rates of increase. As a result, the user can project future high water marks revealing areas under the potential threat of flooding.

The Environmental Protection Agency (EPA) has created a GIS using the United States Geological Survey's (USGS) 1-degree digital elevation series and the National Oceanic and Atmospheric Administration's (NOAA) digital shoreline data to produce a series of maps illustrating coastal areas below the 1.5-meter and the 3.5-meter contours for the eastern United States. The results suggest that approximately 58,000 square kilometers of coastline along the Atlantic Ocean and the Gulf of Mexico lie below the 1.5-meter contour and face the possibility of being flooded within the next two hundred years if sea level rises at a rate of 0.75 meters per century.

A similar GIS has been developed for south Florida that incorporates elevation and land use data to depict those areas with elevations of less than 5 feet and 10 feet. The GIS reveals that significant urban areas in Miami-Dade County could undergo flooding during high tide or storm surge events. A large portion of the Florida Keys have elevations below 5 feet that are at risk of inundation should sea levels increase at the rates predicted by the IPCC and other researchers.

Digital Elevation Models (DEM) and Triangulated Irregular Networks (TIN) also are being used to create three-dimensional GIS of areas that might be at risk as a result of rising sea levels. Three-dimensional visualization of such models allows the user to zoom and rotate the scene from a variety of angles for a better comprehension of the negative impacts associated with sea level rise. This technique can be

especially effective when presenting inundation scenarios to administrative officials and the public.

Once the potential high water areas have been delineated, a GIS can be further developed to integrate a variety of related applications, such as surveys, inventories, monitoring, and modeling. Surveys and inventories of coastal ecosystems, including coral reefs, mangrove forests, and other biota can be incorporated into a GIS to assess those ecosystems at higher risk. Other surveys that are useful for determining vulnerability along coasts consist of population distribution such as residential, commercial, and recreational areas. Similarly, the infrastructure that supports these centers such as power generation and fresh water sources should be added to the GIS.

Efficient monitoring requires the assessment of various coastal data baselines and the evaluation of subsequent alterations in spatial patterns through the use of GIS. Relevant aspects might include changes in coastal land use, wetlands, and shoreline configuration. While monitoring involves real-time components, perhaps the most powerful tool of a GIS is its modeling capabilities allowing the user to simulate sea level rise scenarios while offering complete control over the conditions of the simulations which are made affordable and manageable through the use GIS.

GIS Coral Reef Applications

Numerous environmental organizations already make use of GIS as a principal tool in their protection arsenal. According to the website of the Center for Coastal Monitoring and Assessment (CCMA) at http://ccma.nos.noaa.gov, the mission of the CCMA is to assess and forecast coastal and marine ecosystem conditions through research and monitoring. The CCMA assesses the impacts of climate change on coastal ecosystems through various means such as remote sensing and GIS. Projects include habitat mapping, especially in coral reef environments. The GIS is used to assess the baseline conditions of each coral reef and monitor any changes. Among the goals of the CCMA is to develop a GIS of the benthic habitats of all coral reefs within U.S. waters, including territories as well as an assessment of reef fish ecologies. The CCMA already has mapped essential fish habitats of the U.S. Caribbean and provided color aerial photographs of near shore waters of Puerto Rico and the U.S. Virgin Islands.

However, perhaps the greatest contribution by the CCMA is the production of comprehensive, detailed maps of 2,360 sq km of the shallow-water benthic habitats of the Northwestern Hawaiian Islands. The products include a web site and CD-ROM that provide access to digital GIS data, maps, and satellite imagery depicting the location and distribution of shallow-water coral ecosystem habitats. The GIS maps are characterized by unprecedented detail and represent the first comprehensive assessment of benthic habitats of the shallow water environments of the islands. Efforts also are underway to update the coral ecosystem habitat maps of Florida. The CCMA is an outstanding example of an organization using GIS to provide the best available scientific information for resource managers and researchers as well as offering technical advice and easy accessibility to data.

Similarly, ReefBase is the official database of the Global Coral Reef Monitoring Network (GCRMN), as well as the International Coral Reef Action Network (ICRAN). The ReefBase Project is housed at the WorldFish Center in Penang, Malaysia, with funding through ICRAN from the United Nations Foundation (UNF). According to their website at http://www.reefbase.org, ReefBase gathers available knowledge about coral reefs into one information repository. It is intended to facilitate the analyses and monitoring of coral reef health and the quality of life of reef-dependent people, and to support informed decisions about coral reef use and management.

The website outlines numerous objectives of ReefBase which include developing a relational database and information system for structured information on coral reefs and their resources that will serve as a computerized encyclopedia and analytical tool for use in reef management, conservation, and research. Additionally, ReefBase collaborates with other national, regional, and international databases, and GIS facilities relating to reefs to provide a means of comparing and interpreting information at the global level.

The ReefBase online Geographic Information System (ReefGIS) allows the user to display coral reef related data and information on interactive maps. The ReefGIS offers layers with data related to the location of reefs by type such as barrier reef, fringing reef, patch reef, and shelf reef as well as reef geomorphology and reef depth. Other layers depict coral diseases, coral bleaching, and sea surface temperatures. There is even a layer of reefs that are currently being monitored and the name of the organizations charged with the task.

Among the most noteworthy undertakings by ReefBase is the development of the Millennium Mapping GIS. Using high-resolution Landsat images, ReefBase is collaborating with NASA and other partners to create the first global uniform map of shallow coral reef ecosystems. The full image archive and the GIS shapefiles are available through ReefBase to provide high-quality information about reef location, distribution, and extent.

The Caribbean Reefs at Risk map server at http://coralreefs.nbii.gov/reefsatrisk provides spatial information about coastal areas and human threats to coastal ecosystems for the wider Caribbean Region. Most of the data come from the Reefs at Risk in the Caribbean project sponsored by the World Resources Institute and from the University of the West Indies Caribbean Coastal Data Center. Reefs at Risk supports a GIS map analyzer, which allows interactive viewing of a wide range of spatial data on coral reefs, population, development, physical components, and is capable of modeling threats to the Caribbean reefs.

The Reefs at Risk in the Caribbean project was initiated to raise awareness about threats to coral reefs and to provide detailed information about threats in specific locations across the Caribbean region. These data are available on the map server along with an index of integrated threats. The Reefs at Risk in the Caribbean project also includes an assessment of the economic benefits associated with coral reefs and of economic losses as a result of coral reef degradation. Additionally, the project offers recommendations for preserving coral reef ecosystems.

Conclusion

Long-term studies are needed in order to develop a full understanding of the composition, structure and functioning of coral reefs. Such research includes the monitoring of coral reefs and enables the tracking of changes in the health, species composition, and functioning of these diverse and delicate ecosystems.

The optimal means of managing spatial data is through a GIS, which is capable of producing maps that incorporate numerous layers of interdisciplinary data. Data gathering, modeling, and dissemination through the use of GIS offers a tool for decision-making concerning coral reefs at every level. The small representative sample of organizations and institutions dedicated to coral reef conservation presented in this paper is intended to support the claim that GIS is a powerful tool with extensive spatial data analysis capabilities that can reinforce the process of coral reef protection and policy-making, as well as long-term integrated resource management.

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Biodiversity of reefs: inferring from sparse data

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Abstract. Data on occurrences of a particular organism from publications and museum specimens can be used to infer occurrence of members of that species in places where sampling has not been done and at times in the past and future. Programs to make such inferences are based on knowledge of the habitat correlates of the species and determining where else in the world those habitat parameters occur. Precision of such an inference depends on 1) accurate and precise knowledge of the species' habitat requirements, 2) detailed spatially-explicit environmental data, and 3) comprehensive taxonomic and nomenclatural information. Such inferential tools can be important in understanding biogeographic consequences of climate change, in predicting where invasive species might persist, and in recognizing invasive species.

Key words: biogeographic modeling, habitat requirements, taxonomy.

The problem

Species range maps like those in many field guides (eg that to anemonefishes and their hosts by Fautin and Allen 1992, and that to marine life of southern Africa by Branch et al. 2005), are abstractions of the places where members of the species of concern are known to occur. Although the open Indian Ocean between Sumatra and Sri Lanka is depicted by Fautin and Allen (1992) as being within the range of the sea anemone *Heteractis aurora* (Fig. 1), one could not actually find the animal there because, as Fautin and Allen (1992) point out, these animals are confined to shallow water. Thus they occur along land, in strips so narrow they are not easily indicated on small-scale maps – so the range is represented as covering the entire sea bounded by known or inferred occurrences at the extremes. Local guides portray a smaller portion of the globe, and so can indicate ranges in strips along the coast, as Branch et al (2005) do, but the strips are continuous, and it is likely that members of most species occur in only some of the places indicated, occurrences dictated by the presence of appropriate habitat. Such abstractions over-represent the range of a species.

Maps indicating actual occurrences are typical in technical publications, such as Wallace (1999), and in the growing number of biodiversity websites such as that to sea anemones and relatives (Fautin 2008: eg Fig. 2). With few exceptions, it is likely that members of a species occur in more than the places indicated on the maps, which are where they have been observed or from which they have been collected. These concrete depictions under-represent the range of a species.

A solution

As has been known for many years (Allee et al. 1949), to a first approximation, an organism's range can be inferred from abstracting its habitat characteristics based on places the organism is known to occur, then assuming the organism occurs where its habitat does. Computerized methods make the four-step process of developing such range maps theoretically straightforward. 1) Occurrence records of a species and 2) maps depicting the distribution of relevant environmental parameters are overlain. 3) An algorithm abstracts the values of the environmental parameters that coincide with occurrence records, then 4) maps the occurrence of the environmental parameters within the values known to be compatible with the existence of the organism.

The distribution of these values of environmental parameters is a first-cut inference of the occurrence of habitat suitable for the species and thus the potential range of the species. Modeled environmental data for the past and future allow inferences to be made about historical biogeography and how ranges may shift with climate change.

Many models have been developed to carry out such computations, differing in assumptions and underlying algorithms. Some are referred to as "niche models" but few actually consider biological features other than by proxy with habitat. Sixteen such modeling programs were tested against one another

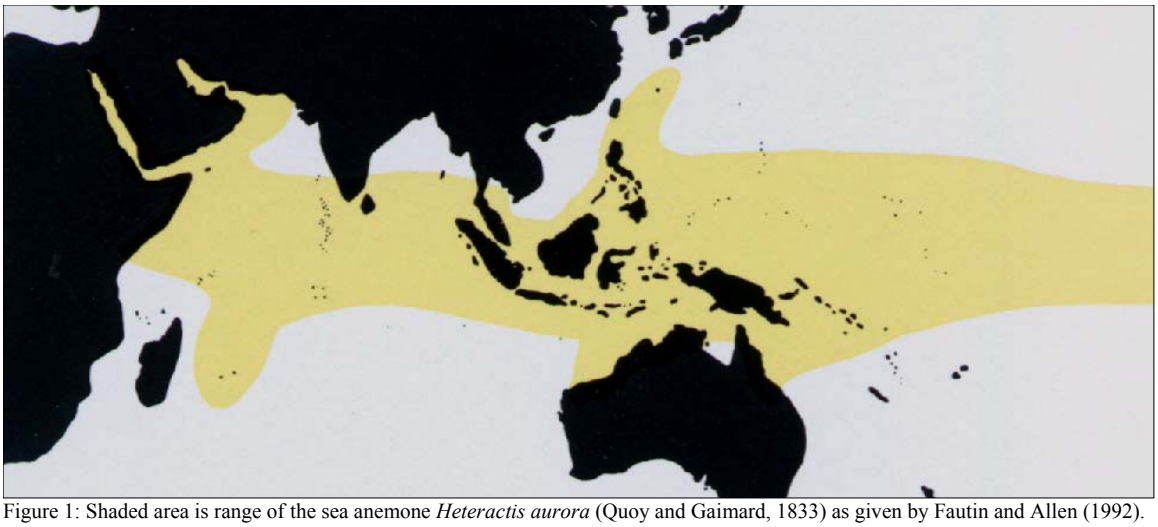

Figure 1: Shaded area is range of the sea anemone *Heteractis aurora* (Quoy and Gaimard, 1833) as given by Fautin and Allen (1992).

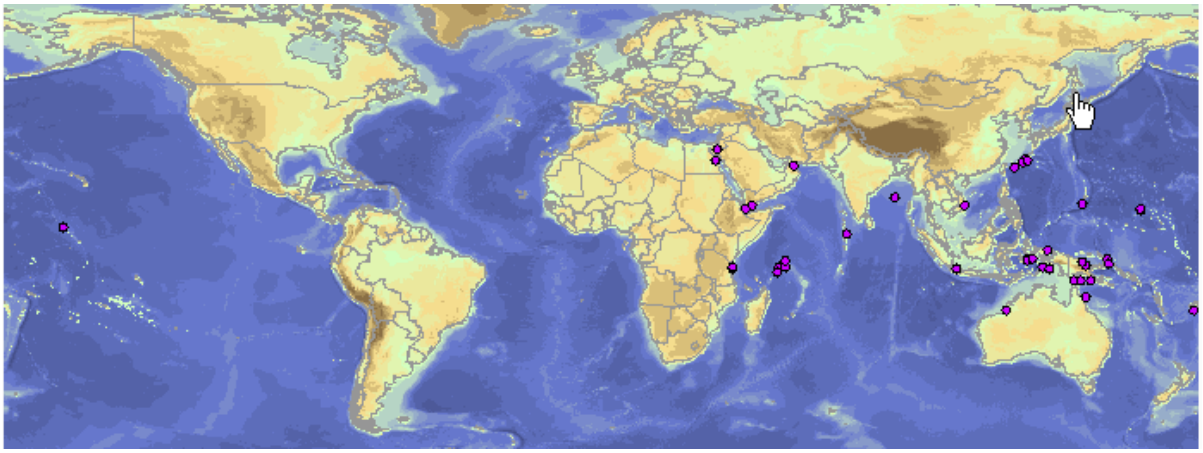

Figure 2: Published occurrences of the sea anemone *Heteractis aurora* (Quoy and Gaimard, 1833) as shown in Fautin (2008).

(Elith et al. 2006), but all were for terrestrial species. One of those programs had been used to model the distribution of some marine fishes, but did poorly for a variety of reasons (Wiley et a.l 2003).

Guinotte et al (2006) and Fautin and Buddemeier (2008) demonstrated the use of KGSMapper, a modeling program that handles marine data well. KGSMapper is associated with 42 spatially-explicit environmental parameters relevant to marine species. Grid size of the associated environmental data is half a degree -- coarse for some purposes – but the coverage is worldwide, which makes it appropriate for many uses. Further, KGSMapper is scale-independent, as demonstrated at "Scleractinian Corals and other Hexacorallians of the Northwestern Hawai'ian Islands" (http://hercules.kgs.ku.edu/hexacoral/hawaii/biodata) in which it is associated with finer-scale environmental data.

Some considerations

Presumably, precision of the environmental data and knowledge of a species' habitat requirements correlate directly with reliability of a range inference. Accuracy of the inference of a species' range should also increase with improved knowledge of the known distribution of the species. One way to maximize information of a species' occurrence is to include all records for the species regardless of the scientific name that was used for it.

Although ideally a single scientific name refers to a single species, many species have been given more than one name (such names are synonyms; International Commission of Zoological Nomenclature 1999). The sea anemone species for which the valid name is *Heteractis aurora* (Quoy and Gaimard, 1833) has been referred to in publication by 11 names (Fautin 2008): it was described as a new species three times, has been placed in nine genera, and has been misidentified.

The points on distribution maps in "Hexacorallians of the World" (Fautin 2008), which depict occurrence records from the published literature, are color-coded by the name used for the species. Because synonyms are a matter of taxonomy – they depend on a

scientist's opinion, and scientists may legitimately differ on whether two names (or more) refer to a single species – this convention allows a user to distinguish among the records by name, disregarding those that the user considers do not refer to the species in question. In fact, such a depiction can be informative in deciding whether a particular synonymy is justified.

Figure 3 is the KGSMapper output of suitable habitat for *Heteractis aurora* based on 75 occurrence records and the environmental parameters mean depth, mean and minimum surface seawater temperature, and maximum and minimum salinity. These parameters were selected because they are relevant to the biology of these anemones. The animals are zooxanthellate, so occur only in shallow water, and are confined to warm, fully saline seas. They are also known only from the Indo-Pacific. Within that natural range, the model output depicts the animal's potential range; this can be used to infer occurrence of members of the species in places where sampling has not been done, which can be useful, for example, in planning fieldwork, or inferring areas into which the animals might expand as climate changes. Outside that natural range (in the Caribbean, for example), the model output can be used to infer where the species might unnaturally occur, as, for example, where it might invade and persist. On the website "Hexacorallians of the World," the darker the reddish color, the more likely the habitat is to be suitable (see Guinotte et al. 2006).

Figure 4 depicts the habitat suitable for *Heteractis aurora* based on the same environmental parameters as for Figure 3, but uses only those occurrence records that referred to the species by one of its synonyms, *Radianthus koseirensis*. Presumably both the smaller number of records and the restriction of them to the western portion of the species' range leads to the conclusion that the species is likely to be

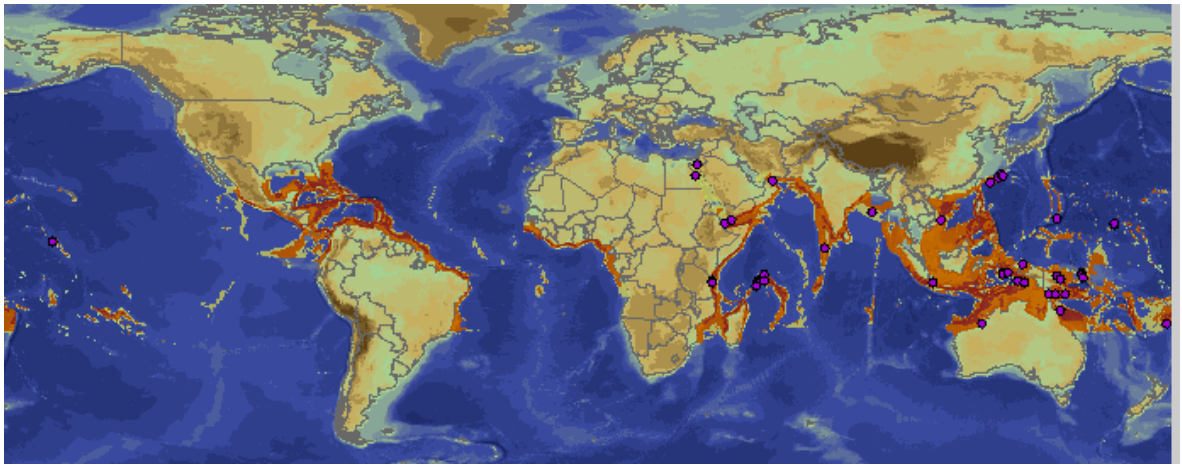

Figure 3: Suitable habitat for *Heteractis aurora* (Quoy and Gaimard 1833) inferred by KGSMapper from 75 published occurrence records (closed circles), and the environmental parameters mean depth, mean and minimum surface seawater temperature, and maximum and minimum monthly salinity. The darker (the reddish color), the more likely the habitat is to be suitable (Guinotte et al. 2006).

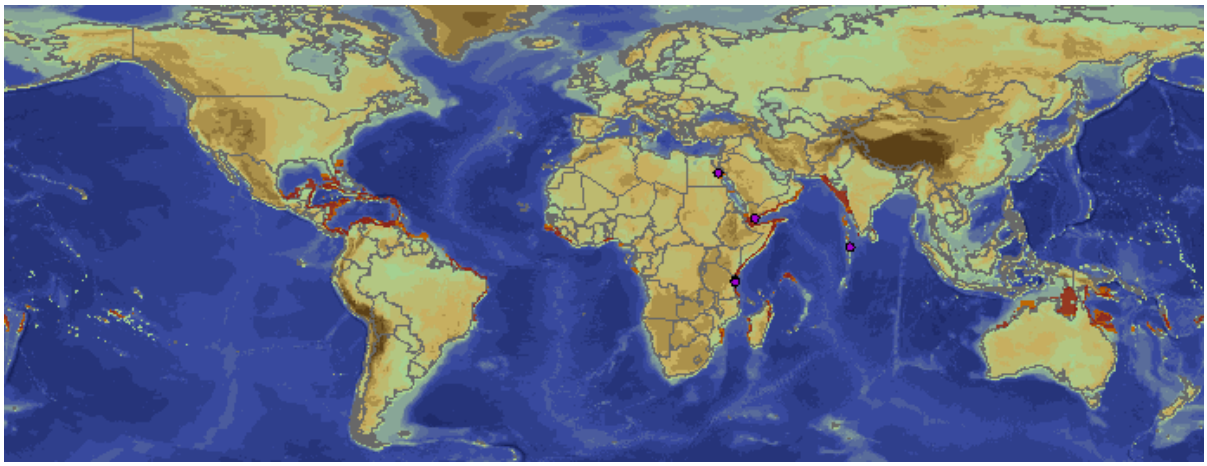

Figure 4: Suitable habitat for *Heteractis aurora* (Quoy and Gaimard 1833) inferred by KGSMapper only from published occurrence records (closed circles) that used the synonymous name *Radianthus koseirensis*. The darker (the reddish color), the more likely the habitat is to be suitable (Guinotte et al. 2006).

far less widespread than is seen in Figure 3. When rendered in black and white, the difference in model output is easiest to perceive in Southeast Asia, where the shaded area of inferred occurrence is more widespread and darker in Figure 3 than Figure 4.

Plotting occurrences using a species name rather than a species concept (by including synonyms) can be not only incomplete but may be misleading. That is because more than one species may have been given the same scientific name (such names are termed homonyms; International Commission of Zoological Nomenclature 1999). Homonyms are not a matter of opinion, but are objectively verifiable, and thus fall within the province of nomenclature. The anemone species to which the name *Heteractis aurora* properly belongs occurs strictly in tropical waters of the Indo-Pacific. That species was first placed in the genus *Actinia* (to which nearly all sea anemones were initially assigned). What is clearly a different species in Britain was also given the name *Actinia aurora*. Applying a model to infer distribution from records that include occurrences of the British species would obviously give a meaningless result.

Conclusions

Use of modeling programs to infer the range of a species from known occurrences and features of the environment benefits from

- accurate and precise knowledge of the species' habitat and natural history (to select environmental parameters relevant to the organism's life that control its distribution)
- knowledge of biogeography (where the organism would be expected to occur, so invasions can be distinguished from occurrences in places where the species might be expected but has not previously been recorded)
- consideration of taxonomy and nomenclature (what other names have been applied to it – and which applications of a name do not refer to the species in question)

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Diversity and abundance of reef macro invertebrates (Mollusca; Echinodermata) in the southern Gulf of California, México

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Abstract. Diversity and abundance of macro mollusks and echinoderms were estimated on three protected islands in the southern Gulf of California. From February 2005 to February 2007, each island was visited twice during warm and cold season. These estimates were calculated from a total of 165 belt transects (25 x 2 m). 22 species of echinoderms and 23 of mollusks were identified. Species richness and abundance showed statistical differences among the islands for both phyla; however, diversity did not. Species assemblages were different on each island. Espíritu Santo had more echinoderms and San José had more mollusks. Asteroids were the most abundant, followed by echinoids and holothurians. The dominant species were the sea star *Phataria unifascialis*, followed by the sea urchin *Tripneustes depressus* and the holothurian *Holothuria fuscoscinerea*. Gastropods were the most abundant with 12 species, whilst bivalves had 7. San José and Cerralvo had more gastropods species than Espíritu Santo. The dominant gastropod and bivalve were *Serpulorbis margaritaceus* and *Pinctada mazatlanica* respectively. The echinoderm and mollusk fauna are similar on the three islands, the community structure is stable along the study area; it seems that habitat type has a strong influence on the distribution and abundance of both phyla.

Key words: Echinoderms, Mollusks, Gulf of California, Abundance, Diversity.

Introduction

The Gulf of California, México, is one of the most important areas for reef species conservation worldwide (Roberts et al. 2002). In the Gulf, the NPA "Islas del Golfo de California" comprises more than 100 islands (Anonymous 1986; 2000a). Many of them support artisanal fisheries and tourism activities thus representing an important economic resource. The islands from the southern Gulf (San José, Espíritu Santo and Cerralvo) are characterized mainly by rocky bottoms which support the presence of isolated coral patches and coral communities, particularly in shallow waters around the islands (1-10 m depth). In this area, coral cover is usually less than 10% (Reyes Bonilla et al. 2005a).

Macro invertebrates, especially mollusks and echinoderms (Asteroidea, Echinoidea, Holothuroidea, Gastropoda, Bivalvia, Opistobranchia) are commonly associated to these communities (Keen 1971; Brusca 1980; Maluf 1988; Cintra Buenrostro 2001; Skoglund 2001; 2002; Solís Marín et al. 2005) and some of them are subjected to local fisheries and some are protected by law (Anonymous 1994). Both phyla play an important ecological role interacting actively with other species and therefore influencing benthic community structure (Benedetti-Cecchi 2001; Chapman 2002). Species assemblages depend greatly on resource availability and on the distance to other populations (Hansky 1991; Woodward & Kelly 2002). However, little is known about the patterns of species assemblages. Even though the mollusk and echinoderm fauna is well known in the Gulf of California (Verrill 1870; Ludwig 1893; Holguín Quiñones et al. 2000; Reyes Bonilla et al. 2005b; González Medina et al. 2006; Herrero-Pérezrul et al. 2008), only a few studies have been directed to ecological aspects of these macro invertebrates. The objective of this study was to analyze the community structure of Mollusk and Echinoderms from San José, Espíritu Santo and Cerralvo discussing some ecological aspects such as abundance, dominance, species richness and diversity.

Material and methods

The islands San José (SJI), Espíritu Santo (ESI) and Cerralvo (CRI) (Fig. 1) were visited twice from February 2005 to February 2007, considering two seasons, warm (Jun-Nov) and cold (Dec-May). A total of 165 (25 x 2 m) belt transects were used to assess the macro invertebrate community on the three islands. Community structure was monitored through the estimation of abundance and frequently used

ecological indexes (Magurran 2004). We calculated the Shannon-Weaner's diversity index (H´), which considers the proportion of the abundance and species richness from the sample and was calculated with the formula:

$$H' = -\Sigma (p_i) (\log_{10} p_i) \quad (1)$$

where: H' = data from the sample in bits/individual; s=species number; p_i = proportion of the total sample in the i-th species.

Data were tested for homoscedasticity using a Kolmogorov-Smirnoff test. A one way ANOVA (α=0.05) was used to compare differences amongst the island's index and used a Tukey test to detect the origin of differences (Zar 1999).

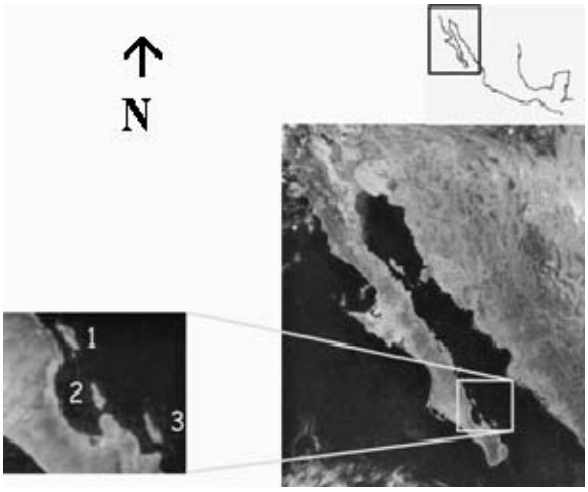

Figure 1. Study area. **1**. San José is located at 25°6'N, 110°31'W; **2**. Espíritu Santo at 24° 4'N, 110°27'W; **3**. Cerralvo at 24°8'N, 109°47'W.

Results

During this study, we counted a total of 5310 echinoderms belonging to 23 species (12 asteroids, 7 echinoids and 4 holothurians) (Table 1); and a total of 2264 mollusks belonging to 22 species (9 gastropods, 7 bivalves and 6 opistobranchs) (Table 2). Species richness of echinoderms and mollusks was not statistically different amongst the islands ($F_{2,26}$= 2.4397, P = 0.09084 and $F_{2,26}$ =1.0643; P =0.36136, respectively). Despite the similarity in richness, the species assemblages were different on each island.

The Class Asteroidea was the best represented on the island complex, followed by Echinoidea and Holothuroidea. The abundance of echinoderms showed statistical differences between the islands ($F_{2,26}$=4.0437, P=0.02958). The Tukey test detected that ESI had the highest values with more than 2500 individuals belonging to the three classes, followed by SJI with almost 2000 individuals, and CRI with less than 1000. At class level, Asteroid abundances also showed statistical differences between the islands ($F_{2,26}$=8.8096, P=0.0012), but holothurians did not ($F_{2,26}$=2.7287, P=0.0942).

The most abundant species was the sea star *Phataria unifascialis,* found ubiquitously along the three islands, followed by the sea urchin *Tripneustes depressus* and the sea cucumber *Holothuria fuscoscinerea*. The commercial holothurian *Isostichopus fuscus* had the lowest abundance (0.07 $\pm$ 0.006 ind/m^2).

Table 1. List of Echinoderm species. * Species observed at the study site but not counted.

Asteroidea	Echinoidea	Holothuroidea
Achanthaster planci	*Arbacia incisa*	*Euapta godeffroyi*
Asteropsis carinifera *	*Centrostephanus coronatus*	*Holothuria fuscoscinerea*
Astrometis sertulifera *	*Diadema mexicanum*	*Holoturia kefersteiini*
Echinaster tenuispina *	*Echinometra vanbrunti*	*Isostichopus fuscus*
Heliaster kubiniji	*Eucidaris thouarsii*	
Linckia columbiae	*Toxopneustes roseus*	
Linckia guildingui *	*Tripneustes depressus*	
Mithrodia bradleyi		
Nidorellia armata		
Pentaceraster occidentalis		
Pharia pyramidatus		
Phataria unifascialis		

Table 2. List of Mollusks species. * Species observed at the study site but not counted.

Bivalvia	Gastropoda	Opistobranchia
*Chama frondosa**	*Cerithium uncinatum**	*Elyisia diomedea*
Hyotissa hyotis	*Chromodoris norrisi*	*Glossodoris sedna*
Nodipecten subnodosus	*Conus sp.*	*Hypselodoris ghiselini*
Pina rugosa	*Murex tricoronis* *	*Navanax aenigmaticus*
Pinctada mazatlanica	*Muricanthus princeps*	*Tambja abdere*
Pteria sterna	*Plicopurpura patula pansa*	*Tambja eliora*
Spondylus calcifer	*Serpulorbis margaritaceus*	
	Strombus galeatus *	
	Thyca callista *	

Regarding mollusks, the community analysis showed that gastropods were the most abundant, followed by bivalves and opistobranchs. The species richness of gastropods showed statistical differences amongst the islands ($F_{2,26}$= 6.8816; P = 0.00503), with the highest value at SJI and the lowest at ESI (Fig. 2). Bivalves were slightly more abundant at ESI and less at CRI, however, no statistical differences were detected ($F_{2,26}$=1.0643; P =0.36136).

The most abundant mollusk was the gastropod *Serpulorbis margaritaceus*, followed by *Muricanthus princeps*. It is important to emphasize the common presence of the mother of pearl *Pinctada mazatlanica*, which amongst the bivalves showed the highest density (0.052 ± 0.03 ind/m^2).

Echinoderms were more diverse than mollusks, but no statistical differences were observed on diversity estimates amongst the islands for both phyla ($F_{2,26}$=2.04, P= 0.3597 and $F_{2,26}$=3.2614, p=0.05448 respectively) (Fig. 3).

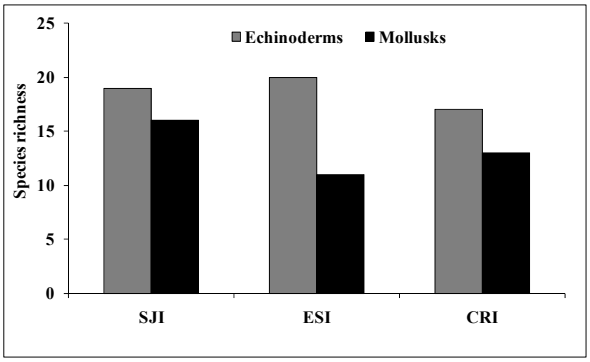

Figure 2. Species richness amongst islands.

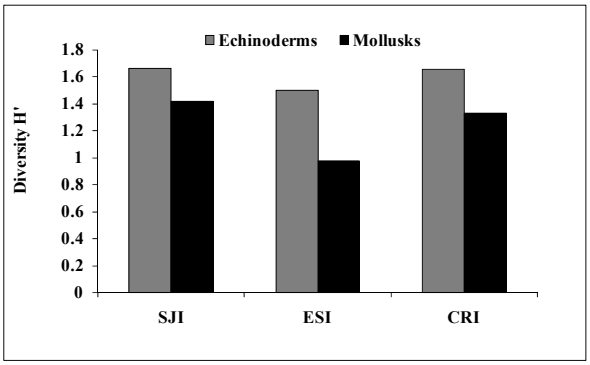

Figure 3. Diversity of echinoderms and mollusks amongst islands.

Species assemblages of echinoderms were different on each island, except for asteroids. The echinoid *Tripneustes depressus* was more abundant in SJI, whilst in ESI was *Eucidaris thouarsii* and in CRI was *Toxopneustes roseus*. Regarding holothurians, *Holothuria fuscoscinerea* was the most abundant and was commonly observed on the three islands, however, *Euapta godeffroyi* was more abundant in ESI and *Isostichopus fuscus* in CRI. Except for the gastropod *Serpulorbis margaritaceus,* the same behavior was observed for mollusks. Gastropods were similarly abundant on the three islands, but bivalves were more abundant in ESI and opistobranchs in SJI. The bivalve *Pinctada mazatlanica* and the gastropod *Muricanthus princeps* were more common at ESI. The images of the dominant species are depicted in (Fig. 4). The holothurian *Isostichopus fuscus* is also shown because of its protection *status* and high importance as an economic resource.

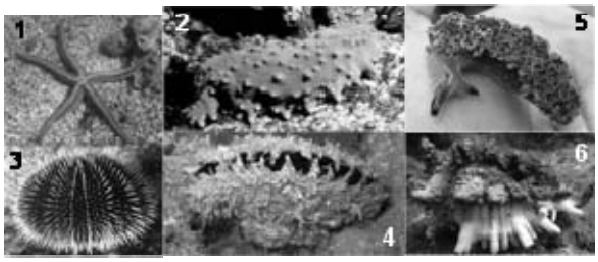

Figure 4. Dominant species at the study site. 1 *Phataria unifascialis*; 2 *Isostichopus fuscus*; 3 *Tripneustes depressus*; 4 *Pinctada mazatlanica*; 5 *Elysia diomedea*; 6 *Muricanthus princeps*.

Discussion

Field visual observations corroborated the number of species of macro mollusks and echinoderms found by census. Since the insular complex involved rocky bottoms, it is possible that some species might have been overlooked due to their habits. Particularly echinoderms were commonly observed hidden in crevices, small caves and between coral branches. Similarly, the gastropod *Serpulorbis margaritaceus* was abundant and frequently counted between coral branches of *Pocillopora* spp. Coral communities and patches from the study area are dominated by *P. verrucosa* and *Porites panamensis* (Gastil et al. 1983; Reyes Bonilla et al. 2005a), and the important role of corals as refuge and substrate for many invertebrates is well known (James 2000, 2006).

The number of macro-echinoderm species varies along the Gulf of California, increasing towards tropical latitudes (Maluf 1988; Cintra Buenrostro 2001), however, it seems that the community structure of echinoderms in the Gulf is relatively homogeneous and is dominated by asteroids (Reyes Bonilla et al. 2005b; Herrero-Pérezrul et al. 2008). At the study site, echinoderms were more abundant and diverse than mollusks. For both phyla, despite the fact that diversity showed no statistical differences among the islands, the species assemblage was different. This is probably related to habitat type. ESI is characterized by a number of shallow bays favoring coral communities and species associated to them. On the other hand SJI and CRI bottoms are mainly composed by boulders and coral communities are less abundant (Reyes Bonilla et al. 2005a).

Regarding echinoderms, asteroids showed the highest value of species richness. It is important to mention that *Phataria unifascialis* was the dominant species, the high values could explain the statistical differences amongst the islands. Some authors consider this sea star as herbivorous (Reyes Bonilla et al. 2005b). During early 1980's the dominant sea star in the Gulf of California was *Heliaster kubiniji*, a

carnivorous sea star, however, in subsequent years, its populations decreased drastically, probably due to the effects of ENSO 1983 or maybe a pathogen (Dungan et al. 1982). *H. solaris* decreased its abundance in the Galápagos Islands after the 1983 ENSO (Hickman 1998). To this date *H. kubiniji* shows no sign of recovery, and it is now considered as rare due to its low densities (Herrero-Pérezrul et al. 2008). It possible that this species shift from carnivorous to omnivorous had some effects on the community structure, however, there is not enough data to support or reject this argument.

On the other hand, we have the presence of *Acanthaster planci*, a well known coral predator. This asteroid had low densities (0.022 ± 0.0016 ind/m^2), it is interesting to notice that it was more commonly observed on rocky bottoms feeding on bryozoans and octocorals. This is easily explained by the fact that in the study area, coral cover is very low (Reyes Bonilla et al. 2005a), thus, there is not enough coral to feed on and it must feed on what is available.

Other important echinoderm is the holothurian *I. fuscus*, which is currently under especial protection by the Mexican government (Anonymous 2000b). The low abundances found in the study site may be caused by legal fishing activity, however, it is possible that these values were also influenced by small (but constant) levels of illegal fishing.

Regarding mollusks, the relatively high abundance of the mother of pearl *P. mazatlanica* is worthwhile to mention. This bivalve was considered an endangered species since early 20th century, and even though data on abundance of wild populations are old and scarce, there is some evidence of an increase on density population (González Medina et al. 2006), and in this study density was even higher that that reported by the authors. It seems that the protection has served its purpose.

One of the most curious mollusk found in this study was the gastropod *Thyca callista*, which parasites *P. unifascialis*. It was not counted on the census, because is mostly hidden on the oral surface of the sea star and is not visible. However, it seems that its presence is common in the Gulf of California (Salazar and Reyes Bonilla 1998, Herrera Escalante 2005). This could be explained by the fact that *P. unifascialis* is more abundant in the Gulf than in the rest of the Pacific coasts of México.

In conclusion, the echinoderm and mollusk fauna are similar on the three islands. The community structure is stable along the study area; it seems that habitat type has a strong influence on the distribution and abundance of both phyla.

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What are the costs of bad taxonomic practices: and what is *Madracis mirabilis*?

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Abstract. The variety of information available today for identification of biological species should allow repeatable and verifiable recognition of the same species by many investigators. However, if good taxonomic practices are not employed, this is not the case. The cost of producing data, which cannot be referred to a described species, is explored here for studies that cite the names *Madracis mirabilis* (Duchassaing and Michelotti 1860) or "Madracis mirabilis sensu Wells 1973". We estimate that in half of such studies published since 1967, specimens named as *M. mirabilis* are not referable to any single species. As such, data from these studies cannot be applied in any analyses that have species-level implications – including studies of diversity, physiology, reproduction, biogeography, ecology and evolution. A rough estimate of the dollar costs of producing these compromised data is just under $4 million dollars. A few basic steps toward improved taxonomic procedures are 1) reference to original materials, including type specimens, original descriptions and study-specific reference materials; 2) a working knowledge and understanding of the international code of zoological nomenclature; 3) maintenance of reference specimens and of records of identification methods and observations; and 4) documenting identification methods within publications.

Keywords: *Madracis auretenra*, *Madracis myriaster*, *Madracis mirabilis*, type specimens, taxonomic code

Introduction

The recent description of a new, but commonly encountered, Caribbean scleractinian coral species, *Madracis auretenra* (Locke, Weil and Coates 2007), raises, again, the high costs of embedded, poor, taxonomic practices. For about 40 years this species has been regularly misnamed as *Madracis mirabilis* (Duchassaing and Michelotti 1860). Mistakes in identification happen, but the errors in evidence here result mostly from using poor taxonomic procedures and represent an unjustifiable waste of expensive, limited, resources. Undertaking only a few, simple procedures would almost guarantee better taxonomy.

For many years *M. auretenra* has been misnamed as *M. mirabilis*. Subsequent to a comprehensive revision by Cairns (1979) of deep-water scleractinians of the Caribbean, some authors named their specimens as "M. mirabilis sensu Wells 1973" (Wells 1973), which Cairns clearly indicated was not the same species as *M. mirabilis* (Duchassaing and Michelotti 1860). *M. auretenra* is shown in Fig. 1. It is a shallower water species, which has zooxanthellae; and it has the apt common name, yellow-pencil coral.

Madracis mirabilis, shown in Fig. 2 is a subjective junior synonym for *Madracis myriaster* (Milne-Edwards and Haime 1849) and *M. myriaster* is a Western Atlantic, deep-water species that does not have zooxanthellae. Type material of *M. mirabilis*

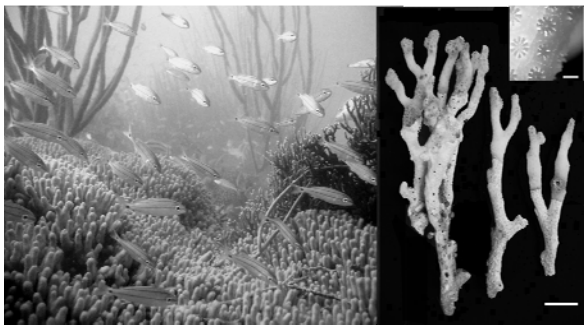

Figure 1: *Madracis auretenra* at Shelly Bay Shoal, Bermuda (left) and holotype (right), from Media Luna SW, Puerto Rico (scale 2 cm), with a close up of the spiny coenosteum (scale 1 mm) (Locke et al. 2007; reproduced courtesy of the Proceedings of the Biological Society of Washington). This species has been misnamed *M. mirabilis* for many years.

and in vivo specimens of *M. myriaster* are shown in Fig. 2. A thoroughly researched synonymy for *M. myriaster* up to 1979 is provided by Cairns (1979); a number of other authors, both before Cairns and more recently, recognized the morphological identity of *M. mirabilis* to *M. myriaster*.

In this paper we present the results of our attempts to discover the identity of specimens that have been named as *M. mirabilis* or as "M. mirabilis sensu Wells" in papers published since 1967. In particular we wanted to know whether these specimens were or were not *M. auretenra*. We also made a rough

estimate of a dollar value for the research effort that might be considered wasted, if no species identity can be resolved. Finally, four simple procedures toward better standards and outcomes of taxonomic practices are provided.

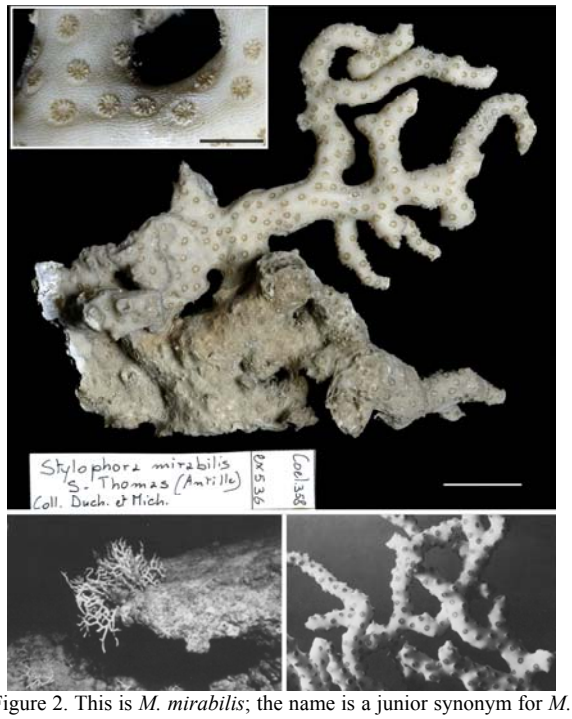

Figure 2. This is *M. mirabilis*; the name is a junior synonym for *M. myriaster*. Type material of *M. mirabilis* from the Zoological Museum of Turin (top) (scale 2 cm). The inset provides a close up of the striate coenosteum (scale 1 cm). Living material of *M. myriaster* from 196 m depth, Castle Roads, Bermuda (Fricke and Meischner 1985).

Methods

Computerized literature searches and visual searches of literature cited sections of relevant publications were made to locate primary references published since 1969 using the species name *M. mirabilis* (Table 1). The first or principal authors of the publications were contacted by electronic mail to see whether they could confirm the identity of their study material as *M. auretenra*, *M. myriaster* or another species, by looking for the distinguishing morphological features that became apparent once *M. auretenra* had been described (Locke et al. 2007). Authors were also asked the source of their current information about their specimens: memory, photographs, reference specimens, or another source.

To estimate a tangible cost of data that are unusable due to incorrect and unrecoverable identifications, we considered the dollar value of time and other resources we have spent describing *M. auretenra*, disseminating that information, and trying to resolve which earlier studies were relevant to *M. auretenra* (Table 2). Then we applied this value to the studies that produced such data. In particular, data that could not be cited in a review of the biology of the new, but very well-studied species, *M. auretenra*, were considered unusable.

Results

The cumulative results of literature searches, queries and responses are shown in Table 1.

Table 1. Use of *Madracis mirabilis* or "M. mirabilis (sensu Wells 1973)" since 1967

Number* of PUBLICATIONS (type 1) naming *M. mirabilis* as a study species (*does not include many regional species lists)	146
PUBLICATIONS (type 2) since July 2007 (post publication of *M. auretenra*) naming *M. mirabilis* (in journals: Coral Reefs, Molecular Ecology Notes, Marine Biotechnology, Marine Biology; and in Coral Reefs of the USA)	6+
Presentations at ICRS 11 naming *M. mirabilis*	**3+**
Number of different first authors of the PUBLICATIONS (type 1, only)	88
Number of investigators to whom *M. auretenra* paper (Locke et al 2007) was distributed (includes some secondary authors)	60
Number of PUBLICATIONS (type 1, only) for which species identities have been verified as *M. auretenra*	60 (41.1%)
Number of PUBLICATIONS (type 1, only) not verified (1. no reply from authors [46 authors], 2. authors could not verify identity [2 authors], or 3. no current contact information for authors [38 publications])	86 (58.9%)
Validation resources reported by responding authors: Reference specimens; Photographs of specimens; Written details of specimens; Personal recollection of specimen details; Field site revisited	

The number of publications naming *M. mirabilis* that we have found, so far, for the period 1967 to July 2007 is 146. More than ten have been published since 2007, including in some prominent publications (such as the journal Coral Reefs, in the new volume "Coral Reefs of the USA" [Riegl and Dodge 2008]), and in the 11th International Coral Reef Symposium abstracts. The 60 publications for which a species identity could be verified included many in which substantial taxonomic information was included, such as close-up photographs of the corallites and coenosteum or detailed descriptions of those structures, or some clear indication by the author(s) that they were not studying *M. mirabilis* (= *M. myriaster*). Eighty-six of the published studies (Table 1) remain doubtful with regard to a species identity. There were three primary reasons why the identity of the specimens could not be or were not verified by the authors of the papers (in descending order of numbers of incidences): no current contact information for the

authors; no response from authors (perhaps because the contact information found was not current); and the authors had no materials or detailed recollections from which they could verify the identity of their specimens.

We prompted authors to indicate the category of resource that they used to verify their morphological species identifications and found that they were mostly as we had expected - reference specimens, good quality photographs, personal, unpublished, descriptions of material, and other personal recollections; however, some authors were also able to revisit their study sites and make new observations of the specimens.

Our evaluation of the time we committed to our studies is shown in Table 2. This is based on actual costs, salaries and in-kind contributions to the project; overhead was both charged against some of the funding for this project and provided as an in-kind contribution, but it is all indicated as overhead.

Table 2. Cost estimate for the *M. auretenra* research project

Expense category		Value USD
Direct costs	Student stipend @ 21,0000/yr*3yr	63,000
	Faculty salary @.10*90,000/yr*3yr	27,000
	Materials and supplies	5,000
	Field work (travel, fuel)	5,000
	Publications	700
	Meeting costs	2,000
Total direct costs		102,700
Overhead	(.30)	30,810
In kind contributions	Lab and field services	5,000
Total costs		138,510

Our expenses related to the publication of the new species *M. auretenra* and to our attempts to widely disseminate information and to affect a correction to the ongoing misidentification of *M. mirabilis* were near $140,000 USD.

Discussion

Data, derived from specimens for which a morphological identity cannot be confirmed, cannot be used in comparative analyses with species-level implications – including studies of: reef ecology, coral physiology, biochemistry, palaeobiology, environmental degradation and global climate change (see Winston 1999 for specific examples). Even if species identity ('getting the name right') is not essential to developing an hypothesis, it still can be very significant whether data from a single species or from a conflated taxon is considered (Weil and Knowlton 1994; Winston 1999). In the case of "M. mirabilis sensu Wells" no description existed for the misnamed "species" prior to 2007 so that authors had very little to refer to as far as producing a consistent identification among different studies. Therefore, it would be hard to justify any assumption that all authors were working on the same, and just one, morphological species.

We know that data from 86 studies, which might be relevant to *M. auretenra*, cannot be included in a review of the biology of that well-studied, widespread, and common species; in fact, at this point those data could not be included in such a study of any coral species. However, the same data may be applicable to very general questions about coral reef ecology and biology. Thus, conservatively, we suggest that only a third of the data produced in those studies has extremely limited value. If we consider that the cost of our studies is in a similar range to the studies we cited and apply a current cost to research over the whole period of the publications we consider, then we would be looking at a total expenditure of about $3,970,620 for data with limited to no value.

This is equivalent to about 57 years of salary for one taxonomic research scientist, with an averaged income through their career of $70,000 per year. This is more than one entire career or equivalent to 30 years salary, a relatively long career, accompanied by an annual research budget of $62,000.

Employing poor taxonomic practices is having significant, long-term, negative impacts on the entire field of coral reef research; both through the negation of data and hypotheses that are used to justify research and conservation protocols and through, simply, wasting large amounts of time and money.

We recommend four easy steps towards better taxonomy, which have been noted in a number of publications on taxonomic procedures, for example, see Winston (1999).

1. Establishing identity:
   - consult original descriptions – electronic copies of original materials are readily provided by most institutions
   - consult with taxonomic experts - question and understand their identification criteria
   - consult type material - types for many Caribbean corals are deposited at the Smithsonian, or other major reference museums in the United States, such as the Peabody at Yale, and at major European museums; they often will lend material or will send good quality images free of charge
2. Understanding naming practices – consult the International Code of Zoological Nomenclature. It is available online at: http://www.iczn.org/iczn/index.jsp
3. Maintaining reference materials

4. Documenting identification methods – record primary and secondary references, comparative methods (material examined), and authorities consulted

The kinds of data available for the identification of scleractinian corals go well beyond the structural features that were the original basis of distinguishing many species. However, no matter what kind or how many data are applied to the question of taxonomic identity, if good taxonomic practices are not applied then good results are not expected.

A review of the biology of the very well-studied coral *M. auretenra* is in preparation. If you have participated in or know of a study that is relevant, please contact J.M. Locke at <janmlocke@yahoo.com>.

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Biodiversity of cryptofauna associated with reefs of the Los Roques Archipelago National Park, Venezuela

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Abstract. Cryptofauna associated with colonies of dead coral is important due to their role as bioeroding organisms that alter the physical structure of reefs. We studied dead colonies of *Montastraea annularis* to obtain information on the main groups present and their relative abundance at Los Roques Archipelago National Park (reefs of Dos Mosquises, Cayo La Pelona, and Cayo Sal). Dead corals were collected at 6-8 m and 9-13 m depth, (3 fragments at each location), and colony volume was estimated for density values standardization. Fragments were broken down in the laboratory and organisms were separated. In all, 1,499 individuals in 8 taxonomic groups were found. The most abundant were sipunculids (57.29%), followed by polychaetes (20.45%), crustaceans (14.76%), nematods, mollusks, and echinoderms. The fauna associated with reefs fragments is highly diverse, but consistently dominated by the same groups.

Key words: cryptofauna, *Montastraea annularis,* dead colonies

Introduction

Coral cryptofauna, initially defined by Klumpp et al. (1988) as mobile organisms using spaces available in coral rock, is made up mainly by a large number of invertebrates. This community of organisms, associated with the reef's calcareous skeleton, inhabits cracks and holes and is classified into borers and opportunists. Borers make cavities in the calcareous layer thus increasing the surface for colonization by organisms such as sponges, bivalves, crustaceans, sipunculans and polychaeta (Hutchings 1974, 1992). Opportunists are unable to bore but take advantage of cavities created by borers. Here one must also include fauna inhabiting the outer surface of the coral, such as fish, crustaceans, echinoderms, and algae. Cryptofauna is has been demonstrated to contribute to the breakdown of the carbonate reef structure in the long run. Few studies in the Caribbean deal with the biodiversity of cryptofauna and none at all concern the southern Caribbean (Ochoa-Rivera et al. 2000; Campos-Vasquez et al. 1999; Moreno-Forego et al. 1998).

The objectives of the present study were to study the biodiversity of crypto fauna associated with dead fragments of *Montastraea annularis*, the dominant species in the region (CARICOMP 1991, Bone et al. 2001), and to evaluate the spatial variations in the distribution of these organisms between various locations on the reef and at different depths.

Material and Methods

This study was executed at Los Roqués Archipelago, (11º 44´45″ - 11º 58´ 36″ N., and 66º 32´ 42″ - 66º 52´ 57″ W; Weil 2003).

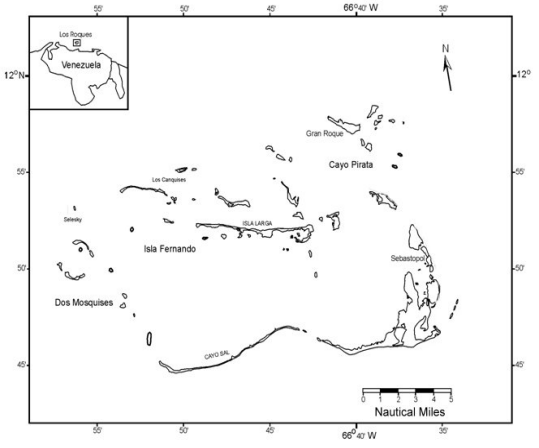

Figure l. Map of Los Roques Archipelago National Park indicating Cayo La Pelona (1), Dos Mosquises Sur (2), and Cayo Sal (3) (Modified from Villamizar et al. 2003). (Fig.1) at two depths (6-8 m and 9-14 m).

Dead fragments *of M. annularis* the second most important coral species in these reefs (14% of total cover; Villamizar et al. 2003) were used. The samples (3 fragments or pillars of *M. annularis*) were taken at Dos Mosquises, Cayo La Pelona, and Cayo Sal. The most exponed and deteriorated pillars were

taken, bringing the total to 18 samples. In the laboratory, coral fragments were broken into smaller pieces with chisel and hammer and organisms were extracted and separated in broad taxonomic groups. Results from the different locations and depths were compared by Kruskal-Wallis analyses ($p<0.05$).

Results

A total of 1.499 individuals were collected, belonging to 8 taxonomic groups, with a mean density of 116±132 ind/l. Sipunculans were dominant, with a relative abundance of 57.3% (mean density of 66±97 ind/l) followed by polychaetes 20.45% (24± 29 ind/l) and crustaceans 14.76% (17±18 ind/l). Chitons, echinoderms, bivalves, gastropods and nematods were also found, but their relative densities was always less than 5% (Table I). The greatest mean density of cryptofauna was found at Dos Mosquises, with 165±216 ind/l, followed by Pelona (131±55 ind/l) and Cayo Sal (51±39 ind/l). Sipunculans always dominated (55.9%, 60.6% and 53.4%), polychaetes were the second most important group in Dos Mosquises and Cayo Sal, with 21.7% and 35.3% respectively. In Pelona, crustaceans (15.6%) were the second group and polychaetes the third, representing 13.09% of the total.

	DMS		PELONA		CAYO SAL		TOTAL	
Invertebrate groups	S	D	S	D	S	D	S	D
Sipunculids	164	21	106	54	28	27	99	34
Polychaets	44	28	9	25	13	23	22	25
Crustaceans	38	14	23	18	3	7	21	13
Bivalves		1	1	2	1		1	1
Ophiuroids	4		1	4		0.2	1	1
Chitons	6	1	3				3	0.2
Nematodes	6	3.0	1.8	4.9		0.29	2.7	2.7
Gastropods	0.2	2	8	2		0.2	3	3
Total	262	69	153	110	44	57	153	79
Total/groups	7	7	8	7	4	6	8	8

Table 1:. Mean density for the invertebrate groups of the cryptofauna for the 3 reef locations (S= shallow, D= deep).

No significant differences were found among the three locations. The shallower zone showed higher density (153±176 ind/l vs. 78±55 ind/l). Sipunculids were more abundant in the shallower zones of all reefs (K-W p: 0.019, Table 1), whereas both groups were dominant in deeper zones.

Discussion

Except for the fact that sipunculids and polychaetes were the dominant cryptofauna at all reefs and all depths, no spatial patterns were noticeable in the distribution, composition, and abundance of cryptofauna. Other studies have also found sipunculids more abundant in the shallower zone (Perry 1998), which could be related to greater biomass, cover and complexity of algae that provide food, substrate and protection (Klumpp et al. 1988), or sipunculid distribution patterns may be more directly related to the degree of dead coral and reef degradation (Peyrot-Clausade and Brunel 1990; Capa 2003; Fonseca et al. 2005). Campos-Vasquez et al. (1999) and Ochoa-Rivera et al. (2000) reported greater polychaete density in the shallow zones of reefs located in Punta Nizuc and Cozumel and explained it by anthropogenic reef degradation. Thus, the lower polychaete densities at Los Roques may indicate good reef health there. Dead coral cover between 14 and 20% reached the maximum values in the shallow zone of La Pelona, where densities of sipunculids were high (Villamizar et al. 2003).

Densities of cryptofauna in this study are low compared to Moreno-Forero et al. (1998), who reported 328 ind/l for polychaetes and 213 ind/l for sipunculids in *Acropora palmata* samples from Colombia. These high values could be related to the state of degradation of the reefs at Isla Grande (Colombia), with <0.2% coral cover. Nonetheless, significant negative correlations were found between cryptofauna density and volume of coral fragments ($r=-0.54$ $p=0.02$), and between sipunculid density and fragment volume ($r=-0.52$ $p=0.029$). Thus more cryptofauna was found in smaller fragments. At Los Roques, high biodiversity of cryptofauna coincided with low abundante and an absence of spatial pattern. Cayo Sal exhibited the lowest diversity.

Studies of cryptofauna thus have the potential of acting as a useful indicador of reef health.

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Comparison of Bacterial Diversity within the Coral Reef Sponge, *Axinella corrugata*, and the Encrusting Coral *Erythropodium caribaeorum*

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Abstract. We compared the Caribbean reef sponge, *Axinella corrugata,* with the Caribbean reef coral, *Erythropodium caribaeorum* for differences in their resident microbial communities. This cursory survey of bacterial diversity applied 16S rRNA gene sequences. Over 100 culture-independent sequences were generated from five different *Axinella* 16S rRNA libraries, and compared with 69 cultured isolates. The culture-independent 16S rDNA clones displayed a higher diversity of Proteobacteria, including "uncultured" or "unknown" representatives from the Deltaproteobacteria. *Arcobacterium*, and Cyanobacteria were also found. We have also confirmed that *Axinella* sponges appeared to host specific microbial symbionts, similar to the previously identified clones termed "OSO" environmental samples. In contrast, seawater samples near *Axinella* were dominated by *Pseudoalteromonas.* Adjacent sediment samples yielded clones of Planctomycetacea, Proteobacteria, sulfate-reducing *Desulfovibrio* spp, and other Deltaproteobacteria. Anaerobe-like 16S rRNA sequences were detected after the oxygen supply to one *Axinella* sample was deliberately curtailed to assess temporal changes in the microbial community. *E. caribaeorum* yielded more Betaproteobacteria relative to *Axinella* 16S libraries, and also included the Gammaproteobacteria genus *Spongiobacter*. However, *Axinella*-derived microbes appeared phylogenetically deeper with greater sequence divergences than the coral. Overall this study indicated that marine microbial community diversity can be linked to specific source hosts and habitats.

Keywords: Sponge, coral, heterotrophic bacteria, 16S rRNA, symbiont

Introduction

Studies of microbial ecology and diversity in the oceans have accelerated over the past decade, partially due to advanced 16S rRNA and metagenomic sequencing methods (Venter et al. 2004; Sogin et al. 2006), plus the recognition of some pivotal microbial functions that include ecosystem services, biogeochemical cycles and symbioses with eukaryotic hosts (Torsvik et al. 2002; Hill et al. 2006; Taylor et al. 2007).

Along these lines, our laboratories have studied marine sponge species for several years. This research includes *Axinella corrugata (Ax)*, a common reef sponge of the Caribbean and Western Atlantic. This bright orange sponge has become a model for natural products chemistry, cell biology, molecular and population genetics (Lopez et al. 2003; Pomponi 2006). Some sponge species have a microbial biomass reaching over 50% (Santavy and Colwell 1990; Fieseler et al. 2004). Similarly, corals have been shown to possess unique and diverse bacterial populations (Rohwer et al. 2001; Ritchie and Smith 2004), but some coral species appear susceptible to bacterial disease outbreaks (Ritchie et al. 2006; Halpern et al. 2008). In addition, the coral, *Erythropodium caribaeorum* (*Ec*) has been shown to produce a wide variety of biologically active diterpenes, such as the anti-mitotic agent eleutherobin (Cinel et al. 1999), and the briarane (erythrolides) and aquariane (aquariolides) skeletal classes (Taglialatela-Scafati et al. 2003).

Longstanding questions regarding the role of microbes in these marine invertebrate hosts, coral reef diseases and potential marine natural product biosynthesis remain. Could coral disease reservoirs exist in other invertebrate species

besides corals, and then jump to reef builders when environmental conditions change (Harvell et al. 2002)? Does the ultimate source of potent natural products stem from resident, symbiotic microorganisms? To provide some baseline data for answering some of these questions we have applied molecular microbiological methods to define the microbial populations associated with these invertebrates and their environments (Sfanos et al. 2005).

Methods

Both coral and sponge hosts are relatively shallow benthic species: *E. caribaeorum* was collected by SCUBA off Fort Lauderdale, Florida at a depth of 30 fsw (feet seawater). *A. corrugata* specimens were collected between 80 – 120 fsw depth off San Salvador and Little San Salvador, Bahamas in 2002. Specimens of *A. corrugata* were held in running seawater for several days to examine short-term temporal changes in the microbial community. One additional specimen was placed in a container with no running seawater to investigate microbial changes under anoxic conditions.

Genomic DNA was rigorously extracted from sponge tissues using a modified guanidium isothiocyanate method (Lopez et al. 2002). Sponge mesohyl (tissue) was obtained from the center of the sponge prior to homogenization Typically 0.5 – 1.5 g sponge mesohyl samples were ground to a fine powder in liquid nitrogen, and incubated for about one hour at 37^{o}C in 5-10 ml of GES (60% [w/v] guanidium isothiocyanate, 20 mM EDTA, 0.5% sarcosyl). DNA from marine sediment samples was extracted using a bead beating method (Mo Bio "UltraClean" soil DNA extraction kit, Solano Beach, CA) according to the manufacturer's instructions.

16S small subunit rRNA sequences were generated by PCR and universal 16S rRNA primers using standard methods previously described (Sfanos et al. 2005). Templates for the cloned 16S rRNA libraries were two different *A. corrugata* specimens. All culture-independent library sponge clones begin with a number, such as "354e", whereas cultured isolates begin with a letter such as T473 etc (also see Sfanos et al. 2005). *Ax* isolates in this study included T295, T266, T274, T288, T473, T273, T280, T456, , T479 S982, and J586 (also see http://www.hboi.edu/dbmr/dbmr_hbmmd.html; Gunasekera et al. 2005). 16S clones derived from *E. caribaeorum* are labeled with "EC".

Sequence and phylogenetic analyses

After confirmation of the closest sequence relative in GenBANK via BLASTN analyses (Cole et al. 2003), new *Ec*-derived sequences were deposited into GenBank and given accession numbers DQ889871-DQ889940, while *Ax* culture independent 16S and isolate sequences had the following GenBank numbers: FJ215389-FJ215423, FJ215474 - FJ215549, and FJ215561-FJ215629.

The program FastGroup II (Yu et al. 2006) was used to perform species richness estimates and rarefaction analyses of individual *Ec* and *Ax* libraries.

Phylogenetic analysis began by aligning sequences using CLUSTALX (Thompson et al. 1997). After manually checking alignments by comparing with known secondary structure models (Sfanos et al. 2005), poorly aligned SSU rRNA regions (e.g. high number of gaps or indels) were omitted from further analysis. Alignments were then imported into PAUP (phylogenetic analysis using parsimony) v 4.0b3a (Swofford 2000), which allowed a comparison of various phylogenetic algorithms and substitution models. Due to the high amount of sequence divergence in most rRNA datasets, minimum evolutionary tree topologies based on distance models were obtained using heuristic methods. Each reconstructed group was statistically evaluated by bootstrapping with a minimum number of 200 replicates (Felsenstein 1985; Nei and Kumar 2000). Most appropriate DNA substitution models for each algorithm were determined using MODELTEST (Posada and Crandall 1998). Reference and type sequences were also downloaded from GenBank in order to help identify specific sequence clusters.

Bacterial Class	***Ax***	***Ax* Sediment**	**Ax Seawater**	***Ec***
Alphaproteobacteria	19	4		15
Gammaproteobacteria	39	9	33	30
Betaproteobacteria				32
Epsilonproteobacteria	1			1
Deltaproteobacteria	22	14		4
Acidobacteria				2
Bacteroidetes	2	2		2
Chloroflexi				1
Nitrospira	1	1		
Planctomycetacea		2		2
Spirochaetes	2			
Verrucomicrobiae		1		1
Cyanobacteria	1			6
Gram positive bacteria	2		2	2
Unknown	21	3	4	
	110	36	39	98

Table 1: Bacterial 16S rDNA clones derived from *A. corrugata (Ax*), *E. caribaeorum* (*Ec*) and environmental samples associated with *Ax.*

Results/Discussion

A total of 110 and 98 16S rRNA clone sequences were sequenced from five different *Axinella* libraries and *Erythropodium*, respectively. Environmental sample libraries were also generated from sediment (39 clones) and water samples (36 clones) collected adjacent to the *Axinella* samples.

Table 1 shows comparative profiles of culture-independent 16S rRNA sequences derived from the two invertebrates and the sediment and water samples collected adjacent to the San Salvador *Axinella* samples.

Among some of the interesting *Ax* sponge-derived sequences, clone 363AM had 96% similarity with an *Arcobacterium*, and several clones had the closest similarity to *Bdellovibrio* and spirochaetes. In addition to the *Arcobacterium*, several additional sequences weakly matching (<90%) to Epsilonproteobacteria were also found in *Axinella,* but are not included in Table 1 at this time, pending verification. A *Nitrospira* clone, 345AU, was also identified. The sponge was further distinguished by the higher proportion of Delta-Proteobacteria and unknown microbial taxa (such as 345 BO, and 345 BM), relative to *Erythropodium.*

The diversity of cultured isolates from *Ax* comprised members of the Alpha-, Gamma- (*Alteromonas* spp*, Pseudoalteromonas* spp. and *Vibrio* spp.) and a few Betaproteobacteria, *Brachybacterium paraconglomeratum* (Actinobacteria) was also found.

The most striking similarity between the two benthic invertebrates, despite geographic and taxonomic separation, was a high representation of the Proteobacteria, especially Gamma-proteobacteria. In contrast, Betaproteobacteria-like sequences were not recovered from the sponge, but represented 32% of all bacteria in *E. caribaeorum.*

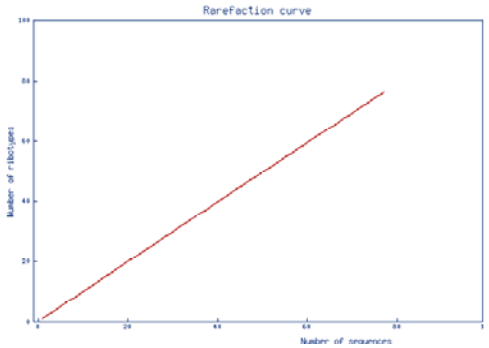

Figure 1: Rarefaction curve of one representative *Axinella* library based on 77 sequences.

About 83% of the *E. caribaeorum* sequences were representatives of the Proteobacteria, 6% of the clones were from cyanobacteria, 2% came from each of the Bacteroidetes, Actinobacteria, Acidobacteria, and Planctomycetes, while ~1% of the clones were represented by each of the *Chloroflexi,* Lentisphaerae and Verrucomicrobia taxa (Table 1).

In the coral, the largest fraction of the Proteobacteria were Betaproteobacteria (32%), followed by Gammaproteobacteria (30%), Alphaproteobacteria (15%), and Deltaproteobacteria (4%). There was only a single Epsilonproteobacteria (Table 1). The genus *Spongiobacter* dominated the Gamma-proteobacteria subdivision in this dataset. Dominating the Betaproteobacteria were representatives of the genus *Aquaspirillum*, followed by a number of clones that had "uncultured or unidentified" designations upon BLAST analysis. Moreover, six *Ec* clones showed high similarity to cyanobacteria sequences.

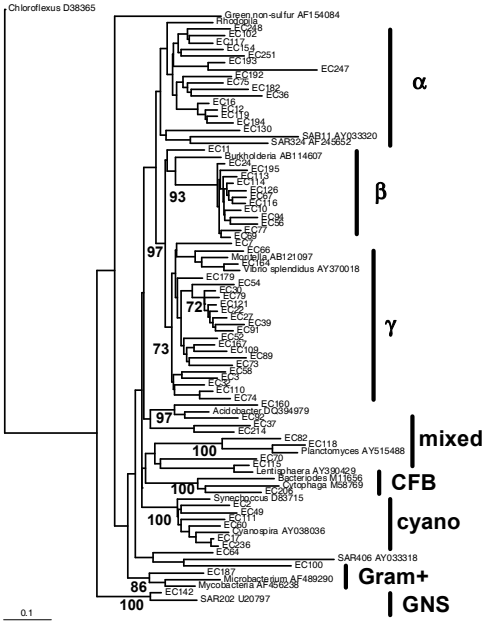

Figure 2: Neighbor-joining tree of *Erythropodium*-derived bacterial 16S rRNA. Reference sequences are listed with their GenBank numbers. Tamura-Nei correct model was implemented. Numbers below nodes are bootstrap percentages after at least 100 iterations. GNS = green non-sulfur bacterial cluster which includes *Chloroflexi.*

The rarefaction curve based on the *Axinella* 16S rRNA sequences shown in Fig. 1, confirmed that sequencing and microbial diversity analyses for this species were not exhaustive. More diversity

may be surveyed by using different 16S rRNA universal primers or DNA extraction methods.

Phylogenetic analyses

Taxonomic diversity within each invertebrate host is partially reflected in the neighbor-joining trees of *E. caribaeorum* and *A. corrugata* 16S rRNA sequences shown in Fig. 2-3, respectively. The analysis was performed primarily to provide a better understanding of taxonomic placement rather than determination of precise phylogenies of bacterial lineages. Nonetheless, the trees highlight the dominance of Proteobacteria in both species. In the *Axinella* tree, triangles denote likely species-specific symbionts (found in >1 specimen). The larger Gammaproteobacteria cluster appeared distinct from other cultured Gammaproteobacteria (e.g. *Vibrio* clade). Several bacteria cultured from

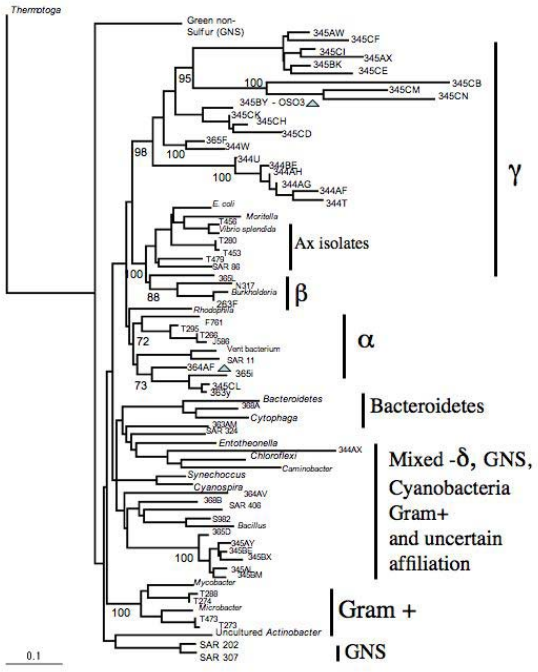

Figure 3: Neighbor-joining tree of *Axinella*-derived 16S rRNA clones. Tree reconstruction parameters and designations follow those shown for *Erythropodium* in Figure 2. A total of 77 bacterial taxa are shown.

Ax ("*Ax* isolates") appear dispersed to either the Gammaproteobacteria cluster or in the Gram+ and Alphaproteobacteria clusters at the bottom of the tree. Not all *Ax* isolates were included in the tree of Fig. 3, but the sequences have been submitted to GenBank. The previous study of cultured sponge isolates of Sfanos et al (2005) showed that most could be identified to family or genus. In contrast, several "unknown" and *Chloroflexi* bacteria were observed from these sponge culture-independent clones.

In the coral, sequences within all Proteobacteria clusters appeared less divergent, with shorter branch lengths, than *Axinella.*

These studies add to the evidence for symbiont specificity in *A. corrugata,* specifically adding to the data on "OSO" ("Orange sponge") studied by Hill et al (2006). OSO 16S rDNA sequences were found in multiple studies of geographically separated *A. corrugata* specimens. The OSO were not found among ~600 other clones generated from other sponge host species (data not shown; Lopez in preparation).

In contrast to the findings with OSO, the Gammaproteobacteria sequences derived from sediments adjacent to *Axinella* samples was uniform and dominated mostly by *Pseudoalteromonas* spp. which were not seen in the mesohyl/tissue libraries of either invertebrate.

Overall, we posit that microbial diversity patterns for both invertebrates generally reflect host species or geography, due to similar depths and water temperature of the specimens. However we cannot completely rule out temporal effects. In a limited time course experiment, some of the *Ax* clones were derived from a specimen that was oxygen deprived; this revealed an anaerobic clone (368B) with similarity to a *Clostridium*.

Although *Bacteroidetes* sequences were detected, this study did not find large occurrences of microbes previously associated with coral diseases such as *Roseobacter* and *Marinobacter* in either host reservoir. However, as the rarefaction analyses indicated, it is very possible that the sampling of total sequences remained below saturation.

It has been demonstrated that many bacterial communities associated with hard corals are largely coral species-specific, with microbial profiles reflecting phylogenetic relationships among coral species (Ritchie and Smith 2004). Previous molecular studies of hard corals have shown that the associated microbiota can be extremely diverse in species richness and abundance (Cooney et al. 2002; Rohwer et al. 2001). The same findings appear to hold for sponges, and other marine invertebrates that can serve as microbial hosts. Although a complete census of marine microbial diversity has certainly not yet been reached (http://icomm.mbl.edu/), microbial profiles are now extended with this study and with the continuing advances in high throughput sequencing technologies (Venter et al. 2004; Rusch et al, 2007).

Conclusions

The present data, in agreement with previous evidence, points to a suite of unique and interesting microbes that appear to be specifically associated with marine invertebrates.

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Diversity of gorgonians and influence of cutting on their growth in the upper Gulf of Thailand

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Abstract. Diversity of gorgonians was investigated in the upper Gulf of Thailand and 15 genera were found. A field experiments determined the influence of cutting of branches on their growth rate. In *Menella,* non-cut colonies grew faster than cut colonies while in *Dichotella,* non-cut colonies grew slower than cut colonies.

Key words: *Menella*, *Dichotella*, gorgonian, diversity, physical disturbance

Introduction

The Indo-Pacific region has a high diversity of gorgonian corals (Goh and Chou 1996; Fabricius and Alderslade 2001; Dautova 2007). Their distribution and abundance are influenced by environmental factors such as light, temperature, water flows, currents, and substrates (Russo 1985; Weinbauer and Velimirov 1996; Zeevi and Benayahu 1999). Azooxanthellate gorgonians usually occur in mid-depth reefs and deep windward fore-reef terrace areas with high currents and sedimentation (Goh and Chou 1995; Goh et al. 1997; Sánchez et al. 1998). Gorgonians also act as refuge habitats for many small invertebrates such as crabs, snails, and brittle stars (Goh et al. 1999; Buhl-Mortensen and Mortensen 2005; Gili et al. 2006). Their shape and their complexity have an influence on diversity and abundance of associated animals (Buhl-Mortensen and Mortensen 2005).

In Southeast Asia, few studies exist on diversity of gorgonians (Alderslade et al. 1989; Goh and Chou 1996; Goh et al. 1997; Ofwegen et al. 2000; Ofwegen and Alderslade, 2007). In Singapore, 12 genera of gorgonians were found (Goh and Chou 1996). In Thailand, gorgonians are common and from previous records 28 genera are known (Alderslade et al. 1989; Worachananant 2000).

In this study, we investigated the diversity of gorgonians in the upper Gulf of Thailand. In addition, the influence of physical disturbance such as cutting on the growth was investigated.

Methods

The surveys were conducted to investigate the diversity of gorgonians at 10 study sites of Mu Ko Samae San, Sattahip, Chonburi Province, the upper Gulf of Thailand (Fig.1). Gorgonians were sampled by scuba diving from 1-20 m depth. Since gorgonians taxonomy needs revision, specimens were only identified to genus level. The density of gorgonians at Laem Pu Chao, western part of Sattahip Bay was investigated with 3 belt transect lines (50 m each) at 6 m depth, where majority of colonies occurred. A diver swam along the line counting gorgonian within 2.5 m on either side.

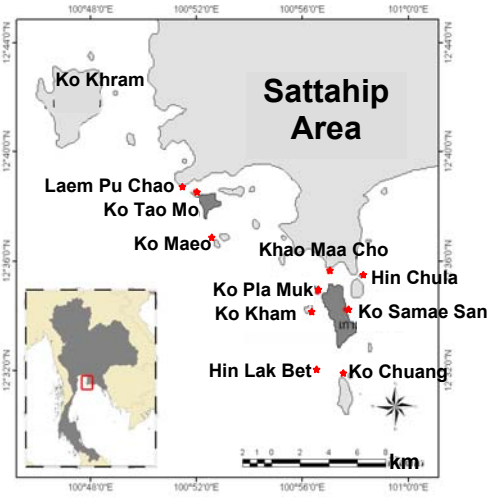

Figure 1: Ten study sites of gorgonians at Mu Ko Samae San, Sattahip, Chonburi Province.

To determine whether physical disturbance such as cutting would influence the growth of gorgonians, field experiments were conducted at Laem Pu Chao. Two genera, *Menella* and *Dichotella* were chosen. *Dichotella* was a dominant genus at Sattahip and *Menella,* although its density was not high in the study area, is a common genus throughout the Gulf of Thailand. Ten colonies of each genus were tagged at 6 m depth. Five of them were control with no

disturbance (normal colony group) while the other five were disturbed by cutting (cut colony group). In each disturbed colony, 5 branches were chosen. Two branches were cut to approx. 5 cm remaining in length (cut branch group) while another 3 branches were left without cutting (normal branch group). For undisturbed colonies, 5 branches of each colony were also selected. Each month, the growth of each branch in both disturbed and non-disturbed colonies were measured. The experiments continued for 16 months.

Results

A total of 15 genera of gorgonians were found at Mu Ko Samae San, Sattahip, Chonburi Province. These included *Subergorgia*, *Melithaea*, *Mopsella*, *Acabaria*, *Euplexaura*, *Echinomuricea*, *Echinogorgia*, *Menella*, *Paraplexaura*, *Astrogorgia*, *Rumphella*, *Guaiagorgia*, *Ctenocella*, *Junceella* and *Dichotella*. Two genera (*Paraplexaura* and *Guaiagorgia*) were the first record found in Thai waters, while three genera (*Echinomuricea*, *Menella* and *Dichotella*) were the first record in the Gulf of Thailand.

Table 1: Diversity of gorgonians found at Mu Ko Samae San, Sattahip, Chonburi Province.

Suborder Family Genus spp.	**Laem Pu Chao**	**Ko Tao Mo**	**Ko Maeo**	**Khao Maa Cho**	**Hin Chula**	**Ko Pla Muk**	**Ko Kham**	**Ko Samae San**	**Hin Lak Bet**	**Ko Chuang**	**Total**
The Scleraxonia Group											
Subergorgiidae Gray, 1859											
Subergorgia	x			x			x				3
Melithaeidae Gray, 1870											
Melithaea	x	x	x	x	x	x	x	x			10
Mopsella							x		x		3
Acabaria		x									1
Suborder Holaxonia											
Plexauridae Gray, 1859											
Euplexaura	x		x					x			3
Echinomuricea	x	x			x		x	x			5
Echinogorgia	x	x			x		x	x		x	6
Menella	x	x	x	x	x		x	x	x	x	9
Paraplexaura		x	x				x	x	x		5
Astrogorgia										x	1
Gorgoniidae Lamouroux, 1812											
Rumphella	x	x	x	x							4
Guaiagorgia		x									1
Suborder Calcaxonia											
Ellisellidae Gray, 1859											
Ctenocella	x	x		x			x				4
Junceella	x	x		x	x		x		x	x	8
Dichotella	x	x	x	x	x		x			x	8
Total (15 genera)	10	11	6	7	6	1	10	6	4	5	

The highest diversity of gorgonians (11 genera) was found at Ko Tao Mo, followed by 10 genera at Laem Pu Chao and Ko Kham (Table 1). The density surveys at Laem Pu Chao showed that *Dichotella* was the dominant genus (59.1% of total gorgonian colonies found) followed by *Ctenocella* (18.4%). *Melithaea*, *Euplexaura* and *Rumphella* had the lowest densities (0.5%) (Fig. 2).

The field experiment on cutting showed that in normal colonies (control group), the percents of monthly relative growth rates of *Menella* and *Dichotella* were 4.7% and 0.6% respectively (Fig. 3). When comparing between cut and non-cut colonies of *Menella,* there was a significant difference in the growth rate (p<0.05). Non-cut colonies had higher percent relative growth rate per month than cut colonies (Fig. 3). However, in *Dichotella,* cut colonies had higher relative growth rate per month than non-cut ones (p<0.05) (Fig. 3). In cut colonies of *Menella*, even though cut branches seemed to grow faster than non-cut branches, there was no significant difference (Fig. 4). In contrast, there was significant difference in the growth rates between cut branches and non-cut branches in the cut colonies of *Dichotella* (p<0.05) (Fig. 4).

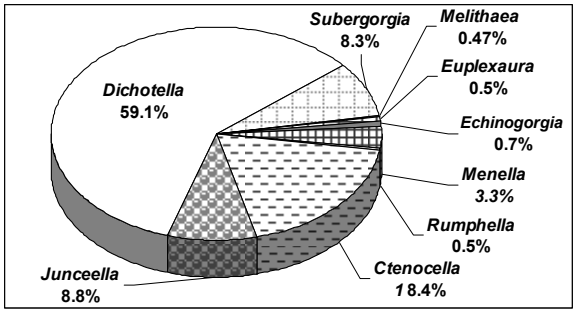

Figure 2: Density of gorgonians in each genus at Laem Pu Chao.

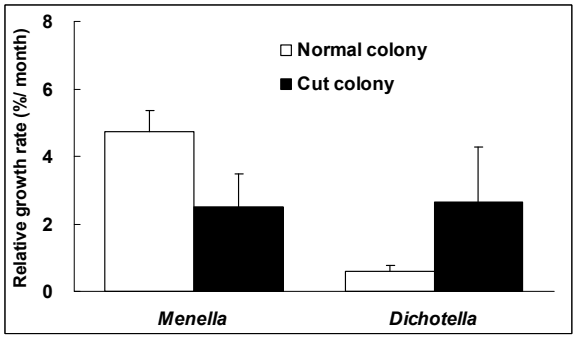

Figure 3: Percent relative growth rate per month of normal and cut colonies in *Menella* and *Dichotella.*

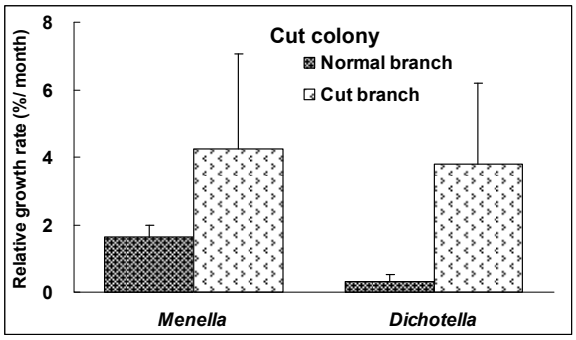

Figure 4: Percent relative growth rate per month of cut and non-cut branches in *Menella* and *Dichotella.*

Discussion

In Chonburi Province, upper Gulf of Thailand, 15 genera of gorgonians were found, a low diversity in comparison with other countries in Southeast Asia. In Indonesia, 225 species of gorgonians were recorded and in the Philippines, 36 genera (Mai-Bao-Thu and Domantay 1970; Grigg and Bayer 1976). Ko Tao Mo had the highest diversity (11 genera). Scleractinian coral species diversity was also high in this area (our observations). This may be due to high currents, one of the major factors influencing the diversity and distribution of gorgonians (Zeevi and Benayahu 1999; Fabricius and Alderslade 2001).

The field experiments showed that reaction to cutting differed between gorgonian genera. In *Menella,* energy needed for the regrowth of the cut branches lowered average growth in comparison to non-cut colonies. In *Dichotella,* cutting stimulated overall growth. More studies are needed for a better understanding of how those factors influence the abundance and growth of gorgonians populations.

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The biogeography of damselfish skull evolution: A major radiation throughout the Indo-West Pacific produces no unique skull shapes

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Abstract
The Indo-West Pacific (IWP) is the center of damselfish biodiversity (Perciformes, Pomacentridae), but phylogeographic evidence indicates that most of the pomacentrids in this region belong to a single lineage that diverged 12-18 million years ago. A strong majority of these species can only be found in coral communities, and this clade represents a major radiation of coral reef fishes within the IWP. Although these fishes constitute approximately half of the damselfishes (183 of 376 species), the results of morphometric analyses indicate they do not possess any unique cranial shapes, and the results of rarefaction analyses reveal that their skull morphology is significantly less disparate than the cranial diversity of the other damselfish clades. The pomacentrid skull shapes that are not represented within this lineage belong to fishes that inhabit rocky reefs. If only species from predominantly coral reef genera are compared, then there are no significant differences in skull shape disparity between these two groups. The Pomacentridae exhibit numerous examples of morphological and trophic convergence, and this tendency towards repeatedly evolving similar ecotypes is exemplified by the finding that a major expansion among the coral reefs of the IWP has produced no unique examples of damselfish skull anatomy.

Key words: Damselfish; Pomacentridae; Functional morphology; Fish feeding; Biogeography

Introduction

The Indo-West Pacific contains the planet's greatest diversity of coral reef fishes (Bellwood and Wainwright 2002a; Carpenter and Springer 2005; Mora et al. 2003) and deciphering the historical patterns of community assemblage in this region is a major goal for marine biologists. The damselfishes represent a significant component of the near-shore reef fish communities throughout the tropics (Bellwood and Hughes 2001; Floeter et al. 2008), and approximately 300 pomacentrid species inhabit the IWP (Allen 1991). In order to understand how the current patterns of IWP coral reef fish diversity have formed, it is therefore necessary to understand the historical biogeography of the Pomacentridae.

Until recently, very few studies have used well-supported phylogenies of large numbers of species to address questions of reef fish biogeography (Bellwood 1997), but fortunately there has been a marked increase in such efforts during the past several years (e.g., Bellwood et al. 2004; Fessler and Westneat 2007; Floeter et al. 2008; Streelman et al. 2002; Westneat et al. 2005). These studies are associated with a recent surge in molecular phylogenetic investigation, and multiple examinations of damselfish relationships have been an important part of this rapidly expanding body of work (e.g., Cooper et al. 2009; Jang-Liaw et al. 2002; McCafferty et al. 2002; Quenouille et al. 2004; Santini and Polacco 2006; Tang 2001; Tang et al. 2004). The most recent of these examines 104 species representing all 28 pomacentrid genera (previously 29), reorganizes portions of the taxonomy, and raises certain questions about the biogeographical history of damselfishes in the IWP (Cooper et al. 2009).

The Pomacentridae can be divided into 5 major clades, each of which now constitutes a separate subfamily (Fig. 1; Cooper et al. 2009). Of these clades, the sister lineages that have diverged most recently are the Abudefdufinae and the Pomacentrinae (Fig. 1). Although relatively recent in origin compared to the rest of the pomacentrid crown group,

the Pomacentrinae have undergone a tremendous amount of diversification, and they now encompass 16 genera, almost exactly half of the damselfishes, and nearly 10 times the number of species as their sister taxon (Fig. 1). Their distribution also spans a massive portion of the globe, with the extremes of their range defined by the eastern coast of South Africa, the Red Sea, the Ryukyu islands of southern Japan, New South Wales and New Zealand, and Easter Island (Allen 1991). Despite the fact that they have radiated over such vast distances, they have not colonized the Atlantic (ATL) or the Eastern Pacific (EP; Allen 1991).

In light of their obvious ability to radiate over large areas that contain warm coastal waters, the absence of the Pomacentrinae from the ATL and EP suggests that their access to these regions has been blocked by dispersal barriers. The most likely of these are the cool ocean waters south of Africa, the geological barrier presented by Africa and the Red Sea landbridge, and the East Pacific Barrier (EPB), which is a large expanse of deep open ocean (~5,000 km) with very little habitat suitable for near-shore fishes (Grigg and Hey 1992). Of these three, only the landmasses can be considered impermeable or "hard" barriers to coastal reef-fish dispersal, while the two purely oceanic barriers in question are "soft" barriers that have been shown to limit, but not prevent, colonization of adjacent ocean regions by reef fishes (Bowen et al. 2006; Briggs 1995; Dı́az de Astarloa et al. 2003; Fessler and Westneat 2007; Floeter et al. 2008; Grigg and Hey 1992; Hadley-Hansen 1986; Lessios et al. 1998; Lessios and Robertson 2006; Robertson et al. 2004; Rocha et al. 2005).

The geological barrier was not in place until the closing of the Tethys Seaway that once connected the ATL and Indian Oceans. This warm water marine dispersal route was blocked by the northward movement of the African and Arabian plates somewhere between 12-18 mya, an occurrence that is referred to as the terminal Tethyan event (TTE; Adams 1981; Bellwood and Wainwright 2002b; Blum 1989). The biogeography of the Pomacentrinae and the Abudefdufinae suggests that they diverged shortly before the TTE, with enough time elapsing between their split and the formation of the Red Sea landbridge for the Abudefdufinae to divide into ATL/EP and IWP lineages (Fig. 1-2; Cooper et al. 2009; Quenouille, et al. 2004). The Abudefdufinae also invaded the ATL/EP a second time, with this colonization occurring more recently, but before the final closure of the Panamanian isthmus 3.1 mya (Coates and Obando 1996). It is possible, but unlikely, that the TTE did not occur until after this second incursion of the Abudefdufinae into the ATL/EP,

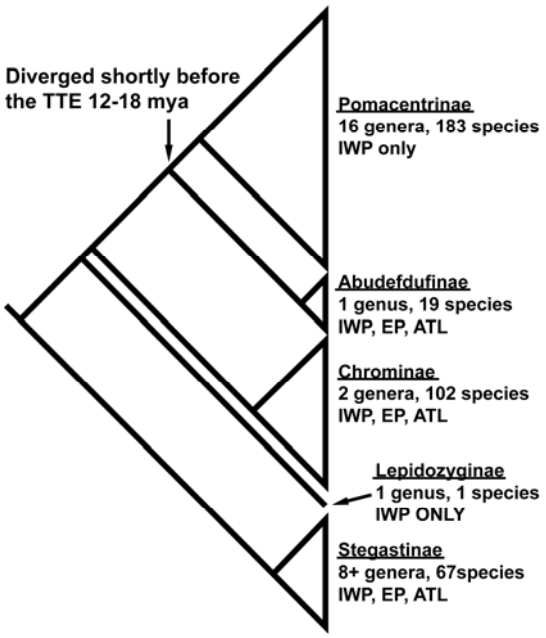

Figure 1. Phylogeography of the damselfish subfamilies. The listing of "8+" genera for the Stegastinae refers to the fact that "*Abudefduf luridus*" lies within this clade, and not with its current cogeners in the Abudefdufinae. IWP = Indo-West Pacific; EP = Eastern Pacific; ATL = Atlanctic. Phylogeny and taxonomy after Cooper et al. (2009).

but such a scenario would make it difficult to explain why the Pomacentrinae failed to invade the same region during this time. A more likely explanation is that the most recently derived species of *Abudefduf* in the ATL and EP colonized this region after the TTE by passing through one of the soft dispersal barriers listed above.

Regardless of the timing of their divergence, the Pomacentrinae represent a major radiation of reef fishes in the IWP that began relatively recently in comparison to the initial divergence of the damselfish crown group. Since the lineage is young (relative to the rest of the Pomacentridae), and since it contains such a large percentage of the damselfish diversity (>48.6 of the species, and >57.0 % of the genera), it is very likely that it has experienced rapid rates of speciation in comparison to the other pomacentrid clades. In order to determine if this lineage's rapid speciation and colonization of a large portion of the world's tropical oceans has been associated with the evolution of derived trophic morphologies, we chose to compare the functional anatomy of pomacentrid feeding between the Pomacentrinae and all other damselfishes.

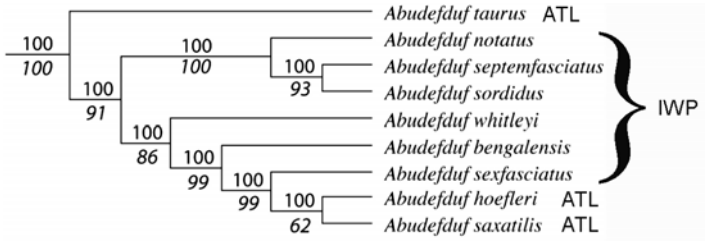

Figure 2. Biogeographic distributions of those species of the Abudefdufinae examined by Cooper et al. (2009). IWP = Indo-West Pacific; ATL = Atlantic. Upper numbers = Bayesian posterior support values. Lower numbers = MP bootstrap support values

Materials and Methods

Specimens

The heads of specimens (N=106) that represent all damselfish subfamilies, genera, and trophic classes (Allen 1999; Allen 1991; Cooper et al. 2009) were dissected in order to expose morphological landmarks of functional importance for fish feeding (Fig. 3). All specimens were adults except in the case of the genus *Altrichthys*, where only juveniles were available. Dissections were digitally photographed in lateral view, and 18 morphological landmarks (LM; Figure 3) were plotted on the image of each specimen using the program tpsDig (Rohlf 2006). This program was also used to determine the Cartesian coordinates of each landmark, and to establish the scale of all images.

Shape analyses

A relative warp (RW) analysis was used to display and quantify patterns of damselfish head shape diversity. The program tpsRelw (Rohlf 2007) was used to perform both a Procrustes superimposition of the data, and a RW analysis of the Procrustes transformed coordinates. Procrustes methods remove differences due to size, orientation and translation without distorting shape information. The RW scores of each specimen were used to map their location on RW axes. Distributions of the specimens in this "shape space" were used to describe relative morphological differences among individual skulls.

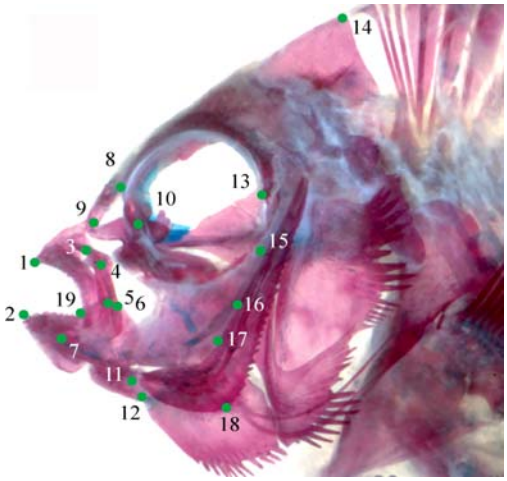

Figure 3. LM examined using RW analysis on a cleared and stained head of *Amphiprion akindynos*.

Comparisons of morphological disparity

In order to statistically compare differences in morphological diversity (shape disparity), a series of 4 different disparity metrics were calculated based on the scores for each specimen on the two largest relative warps (RW1 and RW2). These metrics were: sum of variances, product of variances, sum of ranges and geometric mean range (Stayton and Ruta 2006). Rarefaction of these calculations was then performed using the software application Rarify2 (Stayton 2006) in order to compare members and non-members of the Pomacentrinae using each type of disparity value. All groups were sub-sampled at sample sizes that ranged from 2 specimens to N-2 specimens (N = the number of specimens in each group). All 4 disparity metrics were calculated for 1000 iterations at each sampling level. Since almost all members of the Pomacentrinae are inhabitants of coral reefs (Allen 1991), a second series of rarefaction analyses were performed in order to examine the morphological evolution of only those damselfishes that utilize this habitat.

Results

Inspection of a RW score plot that displays the distribution of damselfish skull shapes along RW1 and RW2 indicates that the diversity of skull form exhibited by the Pomacentrinae is lower than the combined skull shape disparity of the more basal damselfish subfamilies (Fig. 4). The results of rarefaction analyses show that this difference in shape diversity is significant for all 4 disparity metrics at almost every sampling level (Fig. 5). The Pomacentrinae also appear to have evolved no unique skull shapes, since the region of shape space that they inhabit is almost entirely a subset of the region occupied by specimens from the other subfamilies (Fig. 4). Most of this difference is due to the head shapes of relatively large rocky reef inhabitants that evolved within the Abudefdufinae, Chrominae, and Stegastinae, and the exclusion of these specimens from the rarefaction analyses resulted in the elimination of significant differences (Fig. 4-5).

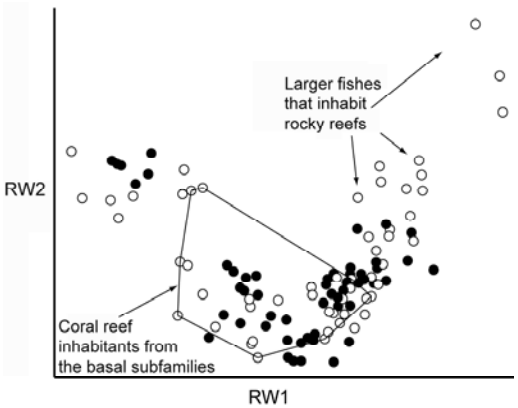

Figure 4. RW score plot of damselfish skull shapes. ●= the Pomacentrinae. ○= specimens from the remaining damselfish subfamilies.

Discussion

The pomacentrinine damselfishes have undergone an extensive, and presumably rapid, increase in species diversity that has not generated any distinct skull morphologies. Since the anatomical landmarks examined in this study are components of the functional morphology of damselfish feeding, our results strongly suggest that this massive IWP radiation has not been associated with an expansion into completely new trophic niches. These findings therefore raise questions about how we can account for the success of the Pomacentrinae.

The results of investigations into the evolution of damselfish skull biomechanics using comparative phylogenetic methods (Cooper and Westneat 2009) indicate that the answers to some of these questions can be provided by observing how frequently damselfishes switch back and forth between a limited number of trophic niches. Almost all pomacentrids feed via herbivory, planktivory, or a limited type of omnivory that very rarely involves preying upon organisms that are highly elusive, durable or difficult to subdue. Although the variety of niches that they occupy is low, pomacentrids are able to quickly transition between them. The evolution of damselfish feeding is therefore characterized by rapid and repeated shifts between this limited set of ecological states, a pattern that has been described as a *reticulate adaptive radiation* (Cooper and Westneat 2009).

This propensity for convergence also applies to pomacentrids at the community level, since those fishes that inhabit coral reefs have repeatedly evolved similar trophic morphologies. The damselfish example, among others (e.g., Adams et al. 2009), demonstrates that evolutionary success need not be limited to lineages that continuously increase their anatomical diversity, but that it can also be achieved by organisms that do a limited number of things, but which somehow manage to do them very well.

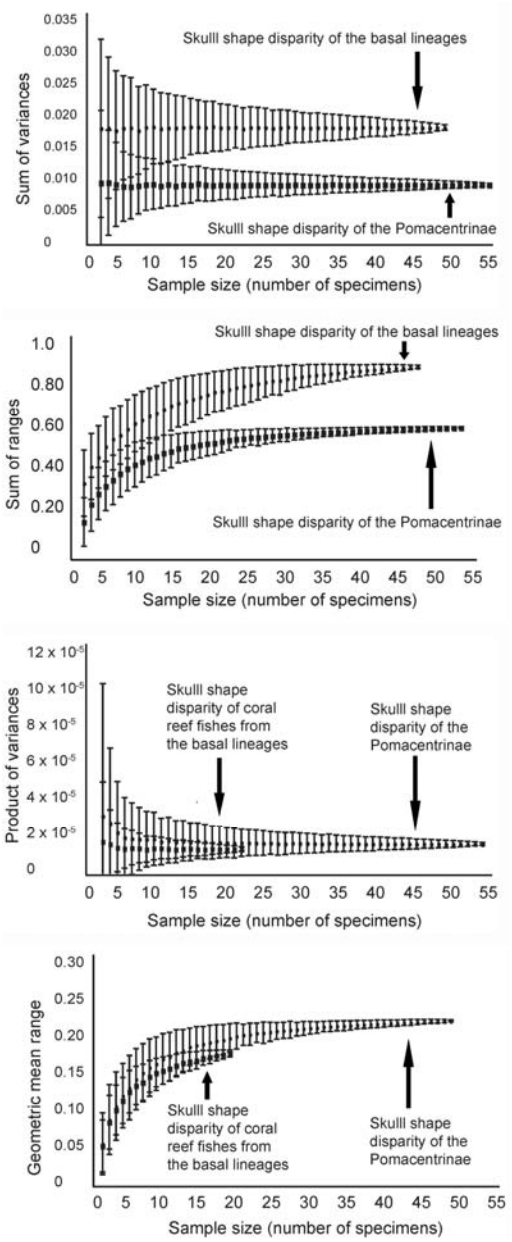

Figure 5. Examples of the rarefaction results.

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Effects of coral mortality on the community composition of cryptic metazoans associated with *Pocillopora damicornis*

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Abstract. Coral reefs consist of complex three-dimensional habitats occupied by diverse taxa. The term cryptofauna (coelobites) specifically applies to those organisms that live within the interstices of coral reef framework. The remarkably diverse assemblage of cryptic biota in reefs is poorly understood relative to that of the epibenthic and nektonic reef species. It has been postulated that the biomass of this cryptic component of the reef ecosystem is high, possibly exceeding that of the surface biota. Furthermore, coelobites play an important role in reef trophodynamics and bioerosion. In order to evaluate the dependency of the cryptic community on the biotic nature of the substrate it occupies, both living and dead *Pocillopora damicornis* colonies were defaunated and returned to the reef. After six and twelve months *in situ*, associated coelobites were collected, identified, counted, and weighed. Communities associated with live coral colonies had greater biomass and were more similar than those associated with dead corals. These findings have important implications for how reef communities may respond to coral mortality.

Key words: cryptofauna, eastern Pacific, coral mortality.

Introduction

Coral reefs are considered to be the most biologically diverse ecosystems in the marine realm (Paulay 1997; Reaka-Kudla 1997). They maintain high biomass and high abundances of organisms despite their occurrence in what are classically considered to be oligotrophic regions. The majority of this concentrated biodiversity (Reaka-Kudla 1997) and biomass (Gischler and Ginsburg 1996) resides not on the surface of the reef but within the cavities of complex structural taxa and framework. Despite their prevalence, these cryptic species are poorly studied compared with the more accessible and charismatic surface fauna (Reaka-Kudla 1997).

Preliminary single taxon (polychaete) surveys have found coelobite densities to be as high as 127,900 individuals m^{-2} (Brock and Brock 1977). Another study found over 107 species living within a single colony of *Pocillopora damicornis* (Grassle 1973).

Although cryptic organisms spend most of their time hidden in cracks and crevices, they are functionally connected to both nektonic species and benthic epifauna. Coelobites are integral components of reef food webs and nutrient dynamics. They provide an essential energy source to many families of marine fish including Balistidae, Cirrhitidae, Haemulidae, Lutjanidae, Labridae, and Serranidae.

Energy, in turn, is incorporated into the coelobite community through a variety of different pathways. Cryptic suspension feeders capture and recycle particulate organic matter from surrounding reef waters (e.g., sponges, lithophage bivalves, polychaetes, barnacles: Richter and Wunsch 1999; Glynn 2008). Cryptic herbivores (e.g., amphipods, chitons, echinoids, limpits, opisthobranchs; Glynn 2008) feed on abundant supplies of algae which are all too often present on modern reefs. Carnivores (e.g., crustaceans, fishes, flatworms, nemerteans, gastropods, octopuses, polychaetes) are also prevalent in the cryptic environment and may exert a high degree of predatory pressure on the community as a whole (Glynn 2006, 2008). Cryptic coral symbionts have been found to benefit coral health and protect their host colonies from predation (e.g, *Trapezia ferruginea* and *Alpheus lottini*; Glynn 1983). Still other cryptic organisms (e.g., lithophage bivalves, polychaetes, sipunculans), play an active role in bioerosion, the biological destruction of coral skeletons and carbonate frameworks. These taxa effectively alter the reef habitat, providing shelters for other opportunistic cryptic species (Hutchings 1983) and ultimately reducing substrate complexity after coral mortality.

The composition of this important coelobite community is thought to be highly dependent on the surface area, volume, and porosity of the substrate it occupies (Hutchings 1974, 1983). Relatively little is known concerning the relationship of the cryptic community with the type of biological substrate it is associated. Some researchers have suggested that coelobite abundances are higher in areas of high algae cover. This may be due to a greater availability of food (Klumpp et al. 1988) or an absence of predation on settling larvae by the coral animal (Hutchings and Weate 1977). Despite these hypotheses, we are not

aware of any study that has directly compared cryptic community composition on living and dead coral.

A large and growing body of evidence has pointed to a worldwide decline in coral cover (Gardner et al. 2003; Bruno and Selig 2007) and increasing occurrence/magnitude of coral mortality events (Glynn 1993). The effect that coral death has on the diverse community of reef taxa is poorly understood. The few studies that have attempted to investigate the effects of coral mortality events have primarily focused on a single taxon (i.e., fishes). Assemblages of fish species have been found to change dramatically within short time periods after mass coral bleaching (mortality) events. Lindahl and others (2001) observed an increase in herbivore abundance and decrease in coral-associate abundance, resulting in an overall increase in fish species diversity. However, over longer time periods, a subsequent loss in habitat complexity may ultimately lead to lower abundance and diversity of fishes (Sano et al. 1987; Booth and Beretta 2002).

Because coral reefs are highly complex and diverse ecosystems, it is important to evaluate the response to coral mortality on multiple taxa assemblages. This study attempts to quantify the difference in community composition of invertebrates associated with live coral (*P. damicornis*) versus those associated with dead coral skeletons (six and eighteen months after mortality).

Material and Methods

Study Area:

This study was conducted at the Uva Island patch reef (7°48'52"N 81°45'34W), located in the non-upwelling Gulf of Chiriquí on the Pacific coast of Panamá. The reef is 2.5 ha and composed primarily of *P. damicornis*. Uva Island experiences a dry season from mid December through April and a wet season throughout the rest of the year. The wet season is characterized by light variable winds, higher rain fall, and higher cloud cover. During the dry season, thermocline shoaling may result in punctuated periods of lower water temperature and elevated nutrient concentrations (D'Croz and O'Dea 2007). Previous research on the Uva Island reef (Abele 1976; Glynn 1982, 1983) and others in the western Pacific (Grassle 1973; Patton 1974; Austin et al. 1980) has demonstrated the importance and prevalence of various taxa associated with the complex arborescent structure of *P. damicornis*.

The Uva Island reef has been extensively monitored by P. W. Glynn and others for the past four decades. Disturbances associated with the El Niño-Southern Oscillation (ENSO) have significantly impacted the area. Elevated water temperatures associated with the 1982-83 ENSO resulted in widespread coral bleaching and 75% mortality (Glynn 1984). The 1997-98, ENSO resulted in only13% coral mortality despite a similar thermal anomaly to the 1982-83 event (Glynn et al. 2001).

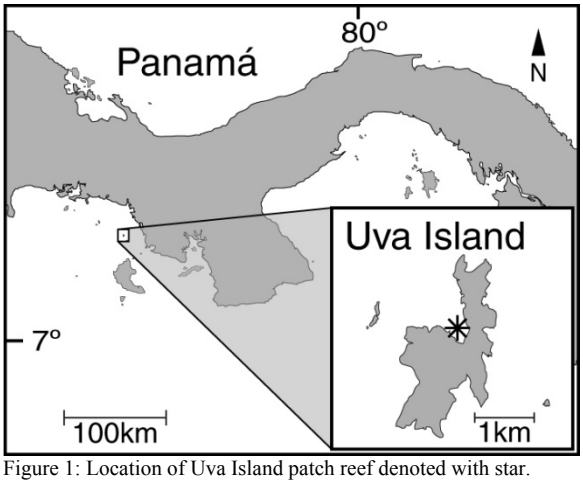

Figure 1: Location of Uva Island patch reef denoted with star.

Field Methods:

In September 2003 sixty colonies of *P. damicornis* were collected from the back reef at the Uva Island site. Maximum and minimum diameter and height were recorded for each colony. All associated metazoans were removed upon collection with aid of forceps and wire probes. Half (30) of the colonies were bleached with fresh water. Necrotic tissue was removed with a high pressure hose, leaving behind bare carbonate skeleton. Crosses were constructed from two pieces of one meter long iron rebar attached at their center. One dead and one living coral colony was affixed to each of two opposite ends of the cross. Live/dead pairs were randomly placed along the fore reef in approximately four to six meters of water.

In March 2004 (six months *in situ*) all experimental units were collected by sealing each colony in a plastic bag underwater. Bags were brought to the surface and each coral colony was defaunated using forceps and wire probes. Seawater from each bag was filtered over a 1mm mesh in order to collect those species that may have come free during collection. Colony dimensions were remeasured and each experimental pair was returned to the forereef. Cryptic metazoans were preserved in 70% ethanol and transported to the University of Miami Rosenstiel School of Marine and Atmospheric Science for further processing.

In March 2005 (12 months since defaunation) 23 of the original 30 experimental units were located and collected. It is likely that after 18 total months *in situ*, significant bioerosion occurred, resulting in the fragmentation and destruction of the missing pairs. Of those that were located, living coral colonies had grown to the point where it was necessary to break them apart in order to collect cryptic fauna. Dead colonies were

also broken apart to eliminate sampling bias. Water from each sampling bag was filtered over 1mm mesh and the dimensions of each colony were recorded. Again, metazoan associates were placed in 70% ethanol and transported to the University of Miami for analysis.

It is important to note that sessile taxa were not cleaned off of dead colonies after the first collection. Therefore, while communities of motile taxa collected in 2005 experienced 12 months *in situ*, the associated encrusting taxa would have developed for 18 months. Additionally, communities of motile fauna collected in 2005 would have experienced both wet and dry seasons during development while those collected in 2004 would have only experienced a single wet season.

Analysis:

Invertebrates were counted and identified to the lowest possible taxonomic level. The bulk wet weight of all individuals of each taxon associated with each colony was recorded. All specimens were deposited in the University of Miami Invertebrate Museum.

The geometric volume of each colony was approximated as a spheroid.

$$V = \frac{4}{3}\pi * \frac{wmax}{2} * \frac{wmin}{2} * \frac{h}{2}$$

Where *wmax* is maximum width, *wmin* is minimum width and *h* is height. Abundances, biomass, and richness were standardized (L^{-1}) by dividing the community parameters for each colony by the theoretical volume of that colony.

Differences in the abundance, biomass, and richness between live and dead colonies were investigated using t-tests and Mann-Whitney rank sum (MWRS) tests when the assumptions of parametric tests were not met. Differences between sampling years were not tested due to the possibility of sampling bias from the use of different methodologies to extract cryptic fauna.

A species by sample abundance (standardized to colony volume) matrix was constructed for each collection year. Abundances were square-root transformed in order to standardize the effects of abundant and rare species on the overall community assemblages. A similarity (Bray-Curtis) matrix was constructed for the transformed abundances using Plymouth Routines in Multivariate Ecological Research (PRIMER 5). The similarity matrix was used to construct an ordination by non-metric multidimensional scaling (nMDS) for each of the two sample years. A cluster analysis (group-average) was also conducted on the same similarity matrix and similarity levels of samples were drawn in the Euclidian space of the nMDS plots.

Results

Abundance:

A total of 1121 individuals were collected from live and dead colonies in 2004 and 4151 individuals were collected in 2005 (Table 1). There was no significant difference in abundance (standardized to volume) between communities associated with live and dead colonies in 2004. In 2005, dead colonies had higher volume-standardized abundances relative to live colonies ($P < 0.01$, MWRS). Proportionally, Crustacea was the most abundant taxon in 2004 (both live and dead) followed in order by Mollusca, Echinodermata, and Polychaeta. In 2005, Crustacea was again the most abundant taxon followed in order by Echinodermata, Mollusca, and Polychaeta. Several common species (mean abundance > 1 L^{-1} in either collection year) were found to be exclusively (e.g., *Alpheus lottini*, *Trapezia* juveniles and *Harpiliopsis spinigera*) or almost exclusively (e.g., *Fennera chacei*, *Pagurus benedicti*, and *Trapezia ferruginea*) associated with live corals. Other abundant species were found to be more closely associated with dead coral (e.g., *Teleophrys cristulipes*, *Palaemonella holmesi*, *Elasmopus* sp., and Gammaridea sp.).

Table 1: The abundance, biomass, and richness of invertebrate communities associated with living and dead *P. damicornis* in 2004 and 2005. Mean values (±SE) have been standardized to one liter by dividing the value for the community associated with each colony by the theoretical volume (approximated as a spheroid) of that colony.

Attribute	2004 (6 months *in situ*, $n = 30$)			2005 (12 months *in situ*, $n = 23$)		
	Live	Dead	Significance	Live	Dead	Significance
Mean Abundance individuals L^{-1}	21.4 (1.6)	22.1 (3.0)	ns MWRS	72.8 (12.2)	135.4 (21.8)	$P < 0.01$ MWRS
Mean Biomass wet weight (g) L^{-1}	3.222 (0.210)	0.820 (0.113)	$P < 0.001$ MWRS	4.224 (0.384)	2.911 (0.584)	$P < 0.01$ MWRS
Mean Richness taxa L^{-1}	8.6 (0.5)	6.3 (0.5)	$P < 0.01$ *t* test	21.3 (1.4)	19.4 (1.2)	ns MWRS

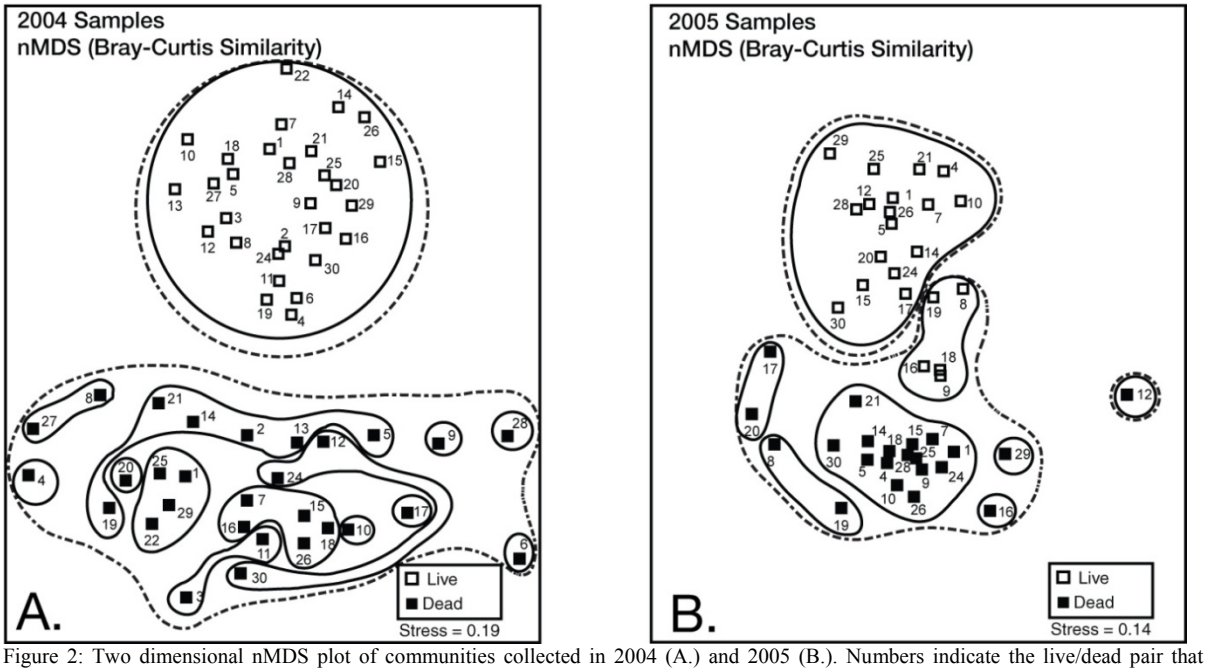

Figure 2: Two dimensional nMDS plot of communities collected in 2004 (A.) and 2005 (B.). Numbers indicate the live/dead pair that each community was associated with. Clusters superimposed at (A.) 22% (dashed line) and 43% similarity (solid line) (B.) 36% (dashed line) and 47% (solid line).

Biomass:

In 2004, 119.3 g of cryptofauna (wet weight) was removed from 30 live/dead pairs. In 2005, a total of 188.1 g of biomass was collected from 23 live/dead pairs. In both 2004 and 2005, the standardized biomass of the community associated with living coral was higher than that associated with dead coral ($P < 0.001$ and 0.01, respectively, Table 1).

Richness:

A total of 117 different taxa were identified (47 to species-level, 43 generic-level, 16 family-level, and 11 higher levels). There was a significantly greater number of taxa associated with living corals in 2004 ($p = 0.002$, *t*-test) but not in 2005 (Table 1).

Ordination:

The nMDS plot of samples collected in 2004 suggests that community composition was more similar between replicates for live coral associated coelobites than dead coral associates (Fig. 2A.). The same pattern was reflected in the ordination of samples collected in 2005. However, a group of five live-associated samples (9, 8, 16, 18, 19) were clustered more closely with dead-associated samples than other live (Fig. 2B.). Communities associated with dead coral in 2005 exhibited a large cluster of greater than 47% similarity which suggests a higher degree of similarity in the 2005 samples than in the 2004.

Discussion

The higher amount of biomass observed in communities associated with living coral colonies suggests a conferred advantage in the form of a greater availability of food and/or protection from predation. Food available to communities associated with a living coral could come from coral fat bodies (Stimson 1990), mucus (Coles and Strathman 1973), or from mucus-trapped plankton (Goldberg 2002). Protection for metazoan associates could be provided from the coral in the form of nematocysts. However, it should be noted that the stinging properties of the corals' nematocysts are ubiquitous and a degree of adaptation would be necessary for a given species to persist in their presence and benefit from their protection.

The qualification that live coral associates must be adapted to cope with the stinging behavior of coral nematocysts may help explain the high degree of similarity observed between live coral communities. Furthermore, several crustacean symbionts (e.g., *Trapezia ferruginea* and *Alpheus lottini*) have been found to be highly territorial and it is likely that they play a role in structuring the community associated with their coral host (Abele and Patton 1976).

The relatively low similarity between many dead coral communities can be explained by higher substrate heterogeneity compared with the live coral habitat. The availability of bare space immediately after coral mortality allowed colonization by a diverse assemblage of fleshy and calcareous algae, sponges, and cyanobacteria. A wider variety of food sources may have led to less specialized, more opportunistic species and consequently greater variability in community assemblages.

This study suggests that coral mortality may directly result in a reduction of cryptic biomass. It is likely that this will lead to a decrease in the biomass of higher trophic groups that directly (invertivore fishes and octopuses) and indirectly (piscivores fishes) utilize the cryptic community as an energy source. The collapse of both predator and prey populations after coral mortality could result in the cessation of important ecosystem functions and ultimately to ecosystem degradation.

It is possible that, given more time, algal growth may be able to support a higher amount of metazoan biomass on dead coral colonies. However, it is more likely that over these longer time scales bioerosion will result in a decrease in habitat complexity and subsequently near to complete cryptic community loss.

Because of the discrepancies in the two sampling years (differences in abundance and richness), it is important to use consistent methodologies to further evaluate seasonal variability and community succession. Furthermore, it is necessary to directly investigate the relationship between encrusting taxa that recruit to dead coral substrate and the motile communities that associate with them. As previously stated, it is likely that post mortality processes (substrate taphonomy, colonization by predator, prey, and territorial species) play in important role in the evolution of cryptic community structure.

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Preliminary Results On Marine Algae Of Madagascar Reef, Yucatan, México: a Functional Group approach.

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Abstract. The abundance and distribution of marine algae was recorded for the first time in Madagascar Reef, a site that may be part of the connection between the Caribbean and the Gulf of Mexico algal communities. The objective of this study is to generate the base-line information on macroalgal functional forms (MFGs) distribution and abundances that can serve to develop a monitoring program for this community. We used the Steneck and Dethier (1994) functional-form system modified. MFGs were examined on 4 sites with three different substrates. The abundance of MFGs and taxa was recorded using a modification of the Braun-Blanquet method. Community differences were analyzed with regard to MFGs and algal taxa distribution. The macroalgal assemblages were found to be variable at the MFG level and widely distributed, except for some of the more complex ones, which tended to be absent in the more continuous rocky substrate. While community analysis at both, MFG and taxa levels revealed similar trends in relation to the type of substrate, we were able to detect important differences in the number of genera that constitute these MFGs. The most diverse site concerning taxa and MFGs composition is probably associated with a more heterogeneous substrate.

Key words: Macroalgae, Functional Forms, Gulf of México.

Introduction

The functional form model is generally used to understand ecological patterns in the diversity of the macroalgal flora. This model is based on the idea that a form-function relationship can be used to interpret distributional patterns in relation to different environmental factors (Littler and Littler 1984). Different models of functional-form groups have been proposed (Littler and Littler 1980; Steneck and Watling 1982; Steneck and Dethier 1994). For this study we used the Steneck and Dethier (1994) system but separated the green calcareous from the coenocytic, as proposed by Collado-Vides et al. (2005) and Phillips et al. (1997), to distinguish conspicuous algae such as *Caulerpa*, *Codium* from *Halimeda* and other cenocytic algae, taking into consideration the local flora composition and morphology.

Madagascar Reef is located between the more structurally complex communities of both the Caribbean and the Gulf of México, therefore the study of this site could help answer questions about connectivity in the flora of the two biogeographical regions. The objective of this study is to generate the base- line information on taxa and macroalgal functional forms distribution and abundances that can serve to develop a monitoring program for this peculiar community.

Material and Methods

Study Area

Madagascar Reef is located at the Northwest of the Yucatan Península, north of the port of Sisal (Fig.1). It forms part of the Inner Campeche Bank Reefs. It is an underwater extension of the Yucatan Peninsula that slopes gradually northwards for about 200 km and descends abruptly into the Sigsby Deep.

Four sites were selected. Site 1 was located at 21°26´26.1´´N and 90°16´55.2´´W, had 24ºC, was 15-17.5m deep and had a sandy substrate; Site 2 was located at 21°26´19.2´´N and 90°16´56.5´´W, had 26ºC, was 7-11m deep and had a mixed rocky-sandy substrate; Site 3 was located at 21°26´19.2´´ N and 90°16´42.3´´W, had 27 ºC, was 7-12m deep and had a rocky substrate; Site 4 was located at 21°26´29.6´´N and 90°17´36´´W, had 24ºC, was 15-17.5m deep and had a rocky substrate.

Field techniques

Between August and September of 2007 four sites were collected along the reef. Two 10m transects marked at 2m intervals were used at each site, with a 25 x 25 cm quadrant placed every two meters (to the

right, center and left of the transect, alternatively) to record the composition and abundance of each MFG (macroalgae functional group). In the field, the abundance of MFGs was recorded using the Braun-Blanquet method, assigning a "relative abundance score" between 0 and 5 in each quadrant (0=absent, 1=few to numerous individuals covering <5%, 2=5-25% cover, 3=25-50% cover, 4=50-75% cover, and 5=75-100% cover). Specimens were collected and taken to the laboratory to determinate taxonomic unit (species or genus) and confirm MFGs.

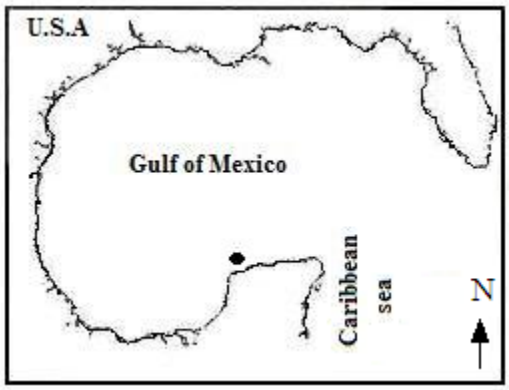

Figure 1: Geographical position of Madagascar Reef.

We used the Steneck and Dethier (1994) system, separating the coenocytic from the calcareous green. For purposes of analyses, data from these quadrates were averaged. Shannon´s diversity index was calculated and matrices with both species and MFGs were built. A percentage of similarity cluster analysis with UPGMA linkage procedure was applied in order to identify similarities in trends between sites, and a PCA was used to determine correlations between sites and species and MFGs using the MVSP 3.1

Results and Discussion

Composition

A total of 55 taxa (visible growth) was recorded (Table 1). At site 1, the most abundant taxa were *Gracilaria* spp, *Halymenia* sp, articulated calcareous algae and Cyanophyta whereas at site 2 the most abundant were *Asparagopsis* and *Dictyopteris* spp. At sites 3 and 4 the Rhodophyta had the highest number of taxa, but *Dictyota* spp and *Dictyopteris* spp (Phaeophyta) had the highest abundance. The least abundant group was the leathery macrophyte constituted by *Sargassum* (Fig. 2).

The 55 taxa were unevenly distributed across the 10 MFGs. The most diversified MFGs were the filaments, corticated polysiphonic filaments and corticated macrophytes, comprising over 50% of the taxa; and the least species-rich group were the foliose and leathery macrophyte, with only one taxon each (Fig.3-4).

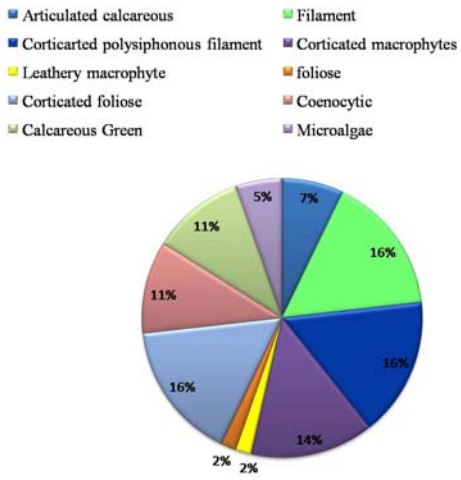

Figure 2: Abundance of different MFG at Madagascar reef.

Macroalgae Functional Groups

Ten MFGs were considered and their generic composition and distribution among sites was as seen in Table 1:

Concerning the abundance of MFGs per site, we found that sites 1 and 2 have eight and nine respectively; corticated macrophytes and the microalgae were the two most abundant at site 1, whereas at site 2 the most abundant were the microalgae, calcareous articulated and filamentous algae. Sites 3 and 4 both have six MFGs, the most abundant being the corticated foliose, the microalgae and the articulated calcareous (Fig. 3).

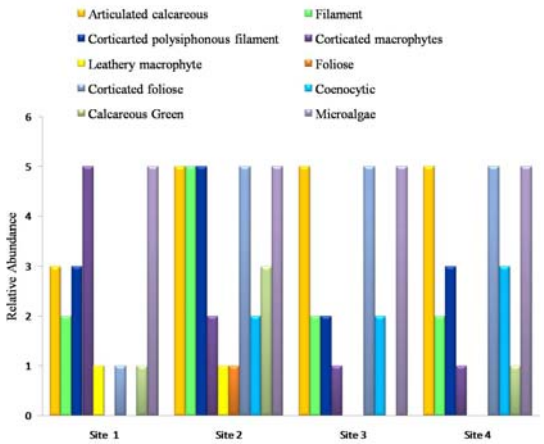

Figure 3: Relative abundance of MFGs per site.

The highest diversity was present at Site 2, both at taxa and MFGs levels, where all except the corticated

foliose group were found. Rocky bottom sites 3 and 4 showed a reduction of both taxa and MFGs, with very few calcareous greens and no corticated foliose algae, foliose and leathery macrophyte (Fig. 4).

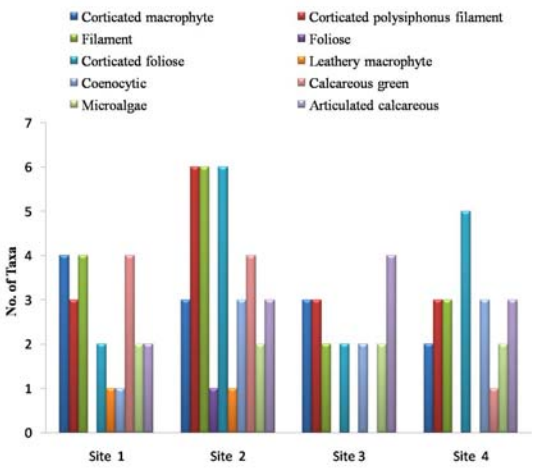

Figure 4: Diversity of Taxa/MGF and MFGs per site.

The PCA data indicate that axis 2 has 98% of the MFG abundance variability. For axis 2, the main component (MFG) that characterized site 1 were corticated macrophytes where *Gracilaria* had the highest abundance. In addition, for site 2 the main component were calcareous green (*Halimeda spp)*, corticated polysiphonous filaments (*Neosiphonia* spp), microalgae (*Lyngbya majuscula*), and filamentous algae (*Asparagopsis* spp.). For axis 1, the main component that differentiates sites 3 and 4 were corticated foliose (*Dictyota* spp), articulated calcareous (*Amphiroa* and *Jania* spp) and coenocytic algae (*Caulerpa* spp) (Fig. 5).

Analysis by MFG

The cluster analysis of similarity percentages indicates that site 3 and 4 had a 94 % similarity, grouping sites with rocky substratum and separating them from sandy and sandy-rocky substrata (Fig. 6).

An algal distribution and abundance pattern was found using MFGs: The macroalgal assemblages were found to be variable at the MFG level and widely distributed, specially regarding the more simple groups such as the filaments and corticated polysiphonous filaments that constitute the turfs, whereas some of the more complex ones, such as foliose and leathery macrophytes, tend to be absent in the more continuous rocky substrate.

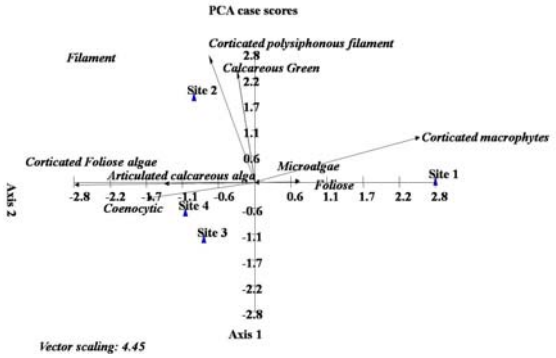

Figure.5: PCA analysis.

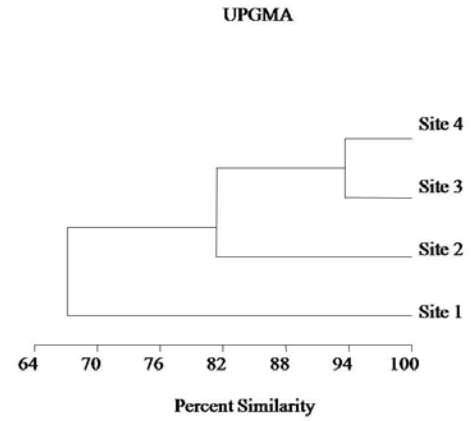

Figure 6: Classification dendrogram of Madagascar reef sites.

The most abundant MFGs at site 1 also belonged to the most abundant taxa (*Gracilaria, Halymenia* and *Lyngbya majuscula*), and the "corticated macrophyte" were the MFGs that covered the most area of the bottom. At site 2, a mixed substrate (sandy-rocky) allowed the presence of more MFGs with high abundance. Sites 3 and 4 had the same three groups with the highest abundance: coralline articulated, corticated foliose and the microalgae, the first two covering almost all the bottom forming a turf composed also in minor proportions by the other MFGs.

To detect any change within a community we first need to understand the spatio-temporal patterns. We still do not know whether the functional form group model provides the correct resolution to detect temporal and spatial changes in community structure, but it helps to provide a first insight on the way the algal community is structured. At this moment the similarity of results of using MFG and "genera" support its use as a rapid and efficient tool to study the spatio-temporal macroalgal distribution and abundance patterns in this type of communities.

Acknowledgement
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Table 1: Composition and distribution of algal taxa per MFG.

Taxa per MFGs per Site	Site 1	Site 2	Site 3	Site 4
Corticated macrophyte				
Agardiella sp				1
Chondria sp		1		
Coelothrix sp		1		
Gelidiopsis sp			1	
Gelidiopsis variabilis	1			
Gracilaria dominguensis	1			
Hypnea spinella	1	1	1	1
Chondrophycus poiteaui	1		1	
Corticated polysiphonus filament				
Ceramium cimbricum		1	1	1
Ceramium cruciatum		1	1	1
Gayliella flaccida	1	1		
Heterosiphonia gibessi		1		
Neosiphonia sphaerocarpa		1		
Polysiphonia binneyi				1
Polysiphonia howey			1	
Polysiphonia sp	1	1		
Spyridia filamentosa	1		1	1
Filament				
Asparagopsis taxiformis		1	1	1
Ptilothamnion speluncarum	1			
Boodleopsis pusilla		1		
Chaetomorpha sp.		1		
Cladophora laetevirens		1	1	1
Cladophora sp	1			1
Rhizoclonium riparium		1		
Sphacelaria rigidula	1	1		
Corticated foliose				
Halymenia floresia	1			
Foliose				
Colpomenia sinuosa		1		
Corticated foliose				
Canistrocarpus cervicornis	1	1	1	1
Canistrocarpus crispatus				1
Dictyota menstrualis		1		1
Dictyota mertensii				1
Dictyopteris delicatula	1	1	1	1
Dictyopteris plagiograma		1		
Dictyopteris polipodioides		1		
Padina sancte-crucis		1		
Leathery macrophyte				
Sargassum hystrix	1	1		
Coenocytic				
Caulerpa brachypus				1
Caulerpa racemosa		1	1	1
Caulerpa verticillata			1	
Caulerpella ambigua				1
Caulerpa webbiana		1		
Codium isthmocladum	1	1		
Calcareous green				
Halimeda discoidea	1	1		
Halimeda incrassata		1		
Halimeda scabra	1			
Halimeda tuna	1	1		1
Penicillus dumetosus	1			
Udotea cyathiformis		1		
Microalgae				
Scytonema sp	1	1		
Oscillatoria sp		1	1	1
Lyngbya majuscula	1		1	1
Articulated calcareous				
Amphiroa fragilisima		1	1	1
Jania adhaerens			1	1
Jania capillacea	1	1	1	
Jania pumilla	1	1	1	1

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Speciation extinction dynamics and the topography of diversity on Indo-West Pacific coral reefs

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Abstract. Profiles of diversity for reef mantis shrimps (Stomatopoda, Crustacea) demonstrate both latitudinal and longitudinal gradients across the Indo-West Pacific (IWP). The diversity summit in the equatorial Indo-Australian Archipelago (IAA) is 2-10 times higher than diversity to the west, east, north and south. From the IAA, diversity declines in the Indian Ocean (IO) but rises toward the African coast (IOC) and declines into the Central Pacific (CP). Endemism is highest in the IAA, high in the western IO and CP, but low in the mid-IO and western Pacific. Small-sized species in the IAA and IOC have restricted dispersal and higher rates of diversification than extinction, producing diversity centers with high endemism. IAA and IOC assemblages also include large species that produce enormous numbers of dispersive larvae, swamping divergence and preventing extinction in IO and western Pacific populations. In the CP, species become increasingly isolated and dwarfed; speciation is high but extinction even higher, yielding low diversity but high endemism. This model explains (1) why diversity gradients occur, (2) why "hotspots" (concentrations of diversity *and* endemism) occur in some but not other taxa (with different life history and dispersal constraints); and (3) why concentrations of endemism do not always coincide with diversity centers.

Key Words: Stomatopod, geographic range, endemism, body size, hotspots

Introduction

Gradients of diversity (species richness) on IWP coral reefs have been explained by the following 9 hypotheses, which range from historical and ecological to artifactual explanations. (1) Present centers of diversity are influenced by faunal carry-over and migration from an older Tethys sea-way center of diversity in Europe (Mesozoic onward; Briggs 2000, 2003a, 2007). (2) Plate tectonics carried antecedent biota and created complex topographic and oceanographic environments that fostered high rates of diversification in the IAA from the Miocene onward (Barber and Bellwood 2005, Barber et al. 2006). (3) Oscillating sea level changes, interacting with a complex topographic and oceanographic environment, changed land mass sizes and circulation patterns, especially during the Pleistocene, and affected marine speciation/extinction dynamics (Potts 1985, Paulay 1990). (4) Currents, originally coupled with the idea of high peripheral origination (Ladd 1960), caused accumulation of species (implying low extinction) in diversity centers (Jokiel and Martinelli 1991, Briggs 1995, Connolly et al.2003, Reaka et al. 2008). (5) The IAA diversity center results from conditions that foster high rates of origination (Center of Origin hypothesis, Briggs 1995, 1999a, 2000, 2003a, 2007). (6) The energy/productivity hypothesis (Rosenzweig 1995) proposes that, over evolutionary time, higher rates of energy flow through an ecosystem allow more species to co-exist (implying lower extinction rates). Viewed as a surrogate of energy input, temperature correlates with rates of speciation across latitudinal gradients (Allen et al. 2006, Allen and Gillooly 2006), but does not explain longitudinal diversity gradients in reef corals and fishes (Bellwood et al. 2005). Higher phytoplankton abundance generally corresponds with greater diversity and species body size in reef-dwelling stomatopods (Reaka 2000, Reaka et al. 2008). However, the rate of energy flow through an ecosystem may be best viewed as predation intensity across a trophic web. (7) Probably acting via species interactions such as predation and competition, species diversity itself correlates with rates of diversification (Emerson and Kolm 2005, Allen and Gillooly 2006). (8) Increased area is often associated with elevated diversity, probably because larger area increases habitat complexity, reducing extinction and allowing more species to coexist (MacArthur and Wilson 1967, Rosenzweig 1995, Losos and Schluter 2000, Bellwood and Hughes 2001, Hubbell 2001, Barber and Bellwood 2005, Bellwood et al. 2005). (9) The mid-domain effect (MDE) posits that random overlap of species ranges which are constrained by geography artificially generates centers of diversity (Woodland 1983; Colwell and Lees 2000; Colwell et al. 2004, 2005; Connolly 2005; McClain et al. 2007).

Although it explains some of the variance, the MDE does not play a major role in forming diversity gradients in IWP fishes and corals (Connolly et al. 2003, Mora et al. 2003, Barber and Bellwood 2005, Bellwood et al. 2005).

The standing crop of diversity at any one place ultimately results from the rate at which species immigrate and either persist, diverge into new species or go extinct. This is essentially the thesis of classical Island Biogeographic Theory (MacArthur and Wilson 1967) and the Unified Neutral Theory of Biodiversity and Biogeography (Hubbell 2001), although the assumptions of these 2 approaches (evolutionary equilibrium under deterministic conditions vs. random ecological drift among species) differ. Except for Jablonski et al (2006), the concept of immigration and speciation/extinction dynamics, however, has not usually been applied to latitudinal and longitudinal diversity gradients, and that is what we explore here.

Materials and Methods

All available IWP distributional records and individual body sizes for species of coral reef stomatopods (Alainosquillidae, Gonodactylidae, Odontodactylidae, Protosquillidae, Takuidae [but not Pseudosquillidae because of their very different reproductive, larval and life history patterns]) were collated, using current taxonomy, from our own and US National Museum collections and the published literature. These data were mapped to determine endemism and diversity. Typical body sizes of species from each region were analyzed according to both maximum and median body sizes of individuals in the population or species assemblage. The 6 biogeographic/habitat regions were chosen on the basis of habitat type (influenced by continents vs. open ocean) and biogeography (cohesiveness of faunas, relative geographic position from the western margin of the IO to the CP).

Results

The Topography of IWP Stomatopod Diversity

We present the first longitudinal transects of species diversity for coral reef stomatopods at 3 latitudes across the IWP (fig. 1). Stomatopod diversity in the IWP is strikingly monolithic—the summit of diversity in the equatorial IAA towers above that of the rest of the IWP in every direction. Less than half as many species are found in the western IO, western Pacific and higher latitudinal regions of the IAA (20°N, 20°S) as in the center of diversity around Indonesia, Malaysia and western New Guinea. Another much lower peak of diversity along the equatorial western margin of the IO climbs higher toward the south around Madagascar. In the IOC, diversities at the equator and 20°S are approximately double those at 20°N. At the West Pacific margin, diversities of the southern and equatorial transects are higher than (but not double) those at 20°N, but in the West Central Pacific, diversities are high only at 20°S in comparison to transects on the equator and 20°N. In the eastern IO (70-90°E) and especially the CP (170-140°W), diversity is strongly attenuated, declining to only 10-20% of that in the equatorial IAA.

Life Histories and Evolution in Stomatopods

Reef stomatopods are limited by the availability of bioeroded holes in the substrate due to intense fish predation (Reaka 1985, Dominguez and Reaka 1987, Steger 1987). Because small holes in the reef are vastly more abundant than large holes (Moran and Reaka 1988), territorial fighting and the possibility of not having a refuge increase—life becomes ever more risky—as species become larger in size. Yet increased body size confers behavioral (mating, fighting) and reproductive benefits.

Within lineages, large species of mantis shrimps grow faster, produce larger and exponentially more eggs with longer dispersal stages, reproduce more frequently, have broader geographic ranges, saturate a higher % of available habitat within their range, compete more intensely for refuges, exhibit more complex fighting and reproductive behavior and are more brightly colored than smaller species (Reaka 1979a,b; 1980, 1985, 1986, 1991; Reaka and Manning 1981, 1987a; Reaka et al. 2008).

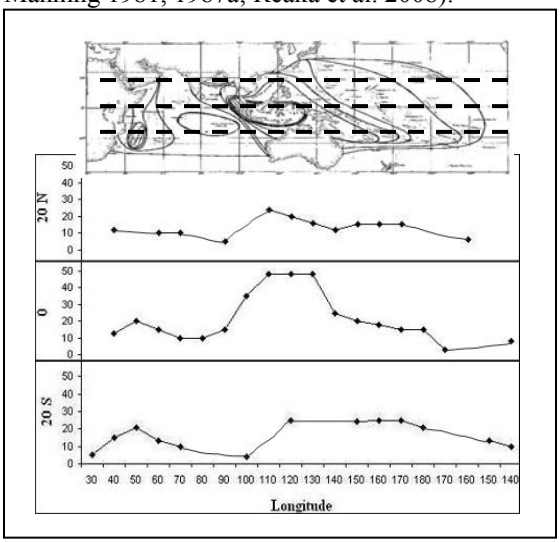

Figure 1. Longitudinal transects of diversity in reef stomatopods at the equator (0°) and at the margins of the tropics (20°N, 20°S). Number of species present at each longitudinal coordinate is indicated on the vertical axis for each transect. Degrees longitude is shown on the horizontal axis. Insect map shows continental masses, contours of diversity across the IWP, and positions of the 3 longitudinal transects (dashed lines).

In contrast, small adult body size is strongly associated with abbreviated larval development and restricted dispersal in marine invertebrates in general (Strathmann and Strathmann 1982; Jablonski and Lutz 1983; Strathmann 1985, 1990; Jablonski 1986a) as well as stomatopods. Small species of stomatopods are characterized by small geographic ranges and high endemism, relative rarity (low abundance, low saturation of available habitat within their range), major ecological innovations and occupation of reefs rather than level bottoms (Reaka 1980, 1986, 1991; Reaka and Manning 1981, 1987a; Reaka et al. 2008).

Because of its close relationship to the life history traits discussed above, body size strongly influences speciation in stomatopods. Large-bodied lineages and those with long-lived larvae remain conspecific or closely related even when separated for several million years by biogeographic barriers (e.g., the Central American Isthmus). In contrast, the % of species without close relatives elsewhere and within locally radiating lineages (more closely related to each other than to any species outside the region) are significantly elevated in small-bodied lineages, those with restricted larval dispersal and those inhabiting reefs. Only taxa of smallest body size within each of the major stomatopod lineages have invaded new types of habitat or adopted new feeding habits (Reaka 1980, Reaka and Manning 1987a).

Similarly, because of its influence upon life history traits and geographic distribution, body size is closely tied to extinction vulnerability in stomatopods. Although adequate fossil data are lacking, apparent extinctions—conspicuous gaps in distributions where extinction is the most parsimonious explanation—are significantly elevated among small-bodied species with restricted larval dispersal. Supporting this interpretation is the fact that, in other groups, species with low dispersal, low abundance and restricted distributions have been repeatedly shown to be more vulnerable to extinction over both ecological and evolutionary time than widespread dispersive taxa (Jablonski 1986a,b, 1987, 1991, 1994, 1995, 2008; Gaston 1994; Gaston and Blackburn 1996; Hubbell 2001; Jablonski et al. 2003; Hunt et al. 2005; Jablonski and Hunt 2006).

Speciation/Extinction Dynamics and Gradients of Species Diversity

Because of the correlation between body size and patterns of life history and evolution in stomatopods (and in many other marine species), body size can be used as a surrogate for the propensity to diversify and become extinct. Figure 2 depicts typical body sizes of reef stomatopod species across the IWP (x^2=11.07, df=5, p=0.02 for species above/below median body size of the 6 assemblages). Although the range in body size of each assemblage is large, especially from the IOC to the IAA, *most* species are diminutive in the diversity centers of the IOC and IAA, suggesting that these regions are hotspots of diversification and extinction. They also are hotspots of endemism (48% and 26% in the IAA and IOC).

Given their life history characteristics, large species in the IAA and IOC undoubtedly swamp the adjacent regions with larvae, accounting for the larger median body sizes of assemblages in the mid-IO and western Pacific regions. Populations in these adjacent off-shore regions are unlikely to diverge, and also are insulated from local extinctions, due to regular influx of larvae from ancestral populations and other large-bodied off-shore populations. Only 12% and 8% of IO and WPM species are endemic.

On the other hand, typical body size declines from the western Pacific regions toward the CP, with associated reductions in number of eggs and dispersal capability. This pattern is particularly exaggerated on atolls compared to high islands (Reaka 2000, Reaka et al. 2008), suggesting that Pacific populations are challenged by low productivity. Given enough time, larvae from large-sized dispersive species in the IAA and western Pacific must occasionally reach the CP, but, once there, the dwarfed populations are trapped, unable to produce enough propagules to reach another archipelago. If the population survives, divergence is almost inevitable due to selection and genetic drift. Endemism increases from 8% to 15% from the WPM to the CP. These founding populations also must frequently become extinct, especially in atoll environments that are characterized by low terrestrial input, high predation, smaller body sizes and lower reproductive capacity than high islands. We have directly observed extinction of 1 such population/species after storm disruption of local habitat on a mid-Pacific atoll (Reaka and Manning 1987b).

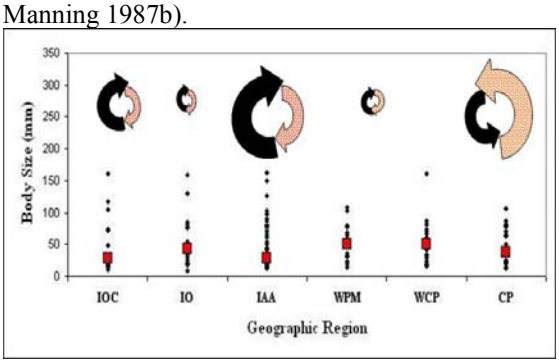

Figure 2. Body sizes of species assemblages of reef stomatopods at each of 6 biogeographic regions in the IWP (IOC=continental margin of the Indian Ocean, IO=open Indian Ocean, IAA=Indo-Australian Archipelago, WPM=western Pacific margin, WCP=West Central Pacific, CP=Central Pacific). Each dot represents maximal size of 1 species, squares represent the median body size of each regional assemblage, size of arrows represents relative strengths of speciation (black) and extinction (stippled).

Therefore, there are 3 centers of diversification in the IWP—in the diversity centers of the IAA and IOC but also in the remote and non-diverse CP. Body size of most species is diminished in all 3 regions, promoting genetic isolation and rapid speciation. However, the causative factors promoting small body size differ in the 3 regions. Biotic interactions (especially competition with other species of stomatopods for bioeroded refuges in the coral) likely are the main factors causing shifts toward predominantly diminutive body sizes (with consequent genetic isolation and rapid divergence) in the productive IAA and IOC. High reef community diversity also may provide more types of minute microenvironments for exploitation by small species in these areas. In contrast, low productivity likely causes dwarfism in the CP. Selection due to low productivity and predation, and especially isolation from western populations, probably are the main factors fostering rapid diversification in the CP.

The processes generating small body sizes and restricted dispersal ability in the IAA, IOC and CP, however, also constrain species to small geographic ranges and render them vulnerable to extinction. High rates of extinction in the IAA and IOC may even fuel higher rates of speciation, given abundant source populations (which is not true in the CP), since extinction continually re-opens niches. This leads to an accelerated cycle of speciation and extinction in the diversity centers. Extinction is especially high, and is likely to overwhelm speciation, however, in the harsh isolated conditions of the CP.

Discussion

Consequently, the standing crop of diversity—as represented in any diversity gradient across a geographic realm such as the IWP—is necessarily a result of 3 processes: immigration, speciation and extinction. Immigration—dispersal from other ancestral and sibling populations—influences both speciation (or lack of it) and extinction (or lack of it). In reef stomatopods, this cycle is controlled by life history traits, especially the number and dispersal capability of propagules produced—which in turn are controlled by species body size—which in turn is controlled by ecological and environmental factors that may vary in different regions, such as along a longitudinal or latitudinal gradient. The standing crop of diversity at any one place is a result of how fast and in what direction the wheels of speciation and extinction are spinning (arrows in fig. 2). In the IOC and especially the IAA, the wheels of speciation/ extinction turn rapidly compared to the adjacent off-shore oceanic regions, and the rate of speciation exceeds that of extinction—yielding high diversity and endemism. The wheels of speciation/extinction also spin rapidly in the CP, but here in the reverse direction—extinction exceeds speciation, yielding low diversity but high endemism. This is *why* concentrations of endemics do not always coincide with areas of high species diversity, *why* "hotspots" (areas in which *both* endemics and high species diversity are concentrated) are not always congruent among different taxa, and *why* a longitudinal diversity gradient stretches across the IWP.

Acknowledgement

The authors thank Christopher D'Elia for pointing out at the ICRS that the patterns of immigration, speciation and extinction we presented resemble the ideas inherent in Island Biogeographic Theory, which we had not explicitly incorporated into our thinking previously.

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Intragenomic ITS2 variation in Caribbean seafans

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Abstract. The internal transcribed spacer 2 (ITS2) is part of the nuclear ribosomal cistron, whose secondary structure has important functions for ribosome assembling. Contrasting results in terms of inter- and intra-specific ITS2 variation have been found in a number of taxa including reef cnidarians. Different findings such as single or multiple intragenomic variants, even pseudogenes, have brought a great deal of confusion regarding the evolution and usefulness of ITS2 for phylogenetic reconstruction as well as the generality of the nRNA concerted evolution process. We examined intragenomic ITS2 copies in Caribbean seafan octocorals *Gorgonia* and *Pseudopterogorgia* (Gorgoniidae: Octocorallia) using Denaturing Gradient Gel Electrophoresis (DGGE) coupled with DNA sequencing and prediction of RNA secondary structures. Candidate pseudogenes were seldom found but most intragenomic ITS2 variants were functional secondary structures. Intragenomic variants were either dominant or codominant banding patterns in DGGE gels. Preliminary phylogenetic analyses showed that part of the intragenomic variation in *G. mariae* grouped partially with *Pseudopterogorgia* spp. as well as with other *Gorgonia* species. This finding supports the polyphyly of Caribbean sefans as observed with mitochondrial DNA and suggests a likely hybridization origination for *G. mariae*.

Key words: Intragenomic variation, ITS2, rDNA, Caribbean seafans, *Gorgonia*, hybridization, DGGE.

Introduction

The Internal Transcribed Spacer 2 (ITS2) is part of the ribosomal DNA cistron, which is transcribed but do not form part of the functional ribosomal complex. Although ribosomal genes are sequences repeated hundred of times in several chromosomes there are mechanisms that homogenize all the ribosomal genes (Elder and Turner 1995). Concerted evolution in ribosomal genes, although not entirely understood, is supposed to occur in a few generations owing a combination of processes such as intrachromosomal homogenization, gene conversion and unequal crossing over (Liao et al. 2000). Consequently, all copies of ribosomal genes are usually identical and can be considered single-copy genes for phylogenetic purposes. Nonetheless, ribosomal DNA intragenomic variation has puzzled molecular systematists and ecologists in the last few years.

The ITS2 attains higher evolution rates than other ribosomal genes. In addition, ITS2 has exhibited intragenomic variation in organisms ranging from plants to vertebrates. For that range of eukaryotes the intragenomic variation has been related to hybridization events (Coleman 2003). In the particular case of corals, however, it has been very difficult to explain the presence of intragenomic variants including a range of competing explanations such as incomplete lineage sorting, resulting in the preservation of ancestral polymorphisms, and introgressive hybridization (Volmer and Palumbi 2004). Opportunely, ITS2 is gaining credibility very fast. Coleman (2007), Schultz (2005) and Muller et al. (2007) are examples of some recent reviews showing generalities shared by all eukaryotes on several hallmarks of the ITS2 secondary structure, which have provided robustness using this sequence in phylogenetic and evolutionary studies. The function of the ITS2 secondary structure, unknown for many years, has a very important role during the ribosomal assembling. It is known experimentally that certain changes in the secondary structure prevent the formation of ribosomes (Cote and Peculis 2001, Van Nues et al. 1995). In addition, the same ITS2 sequence can turn into two different secondary structures, where the proximal part does not vary and it has an important function such as the C2 processing site and other parts of the molecule have a multifunctional role. Having that in mind, we can rely upon secondary structure to tell apart pseudogenes from functional ITS2 copies.

Seafans are shallow-water octocorals, which are very abundant in Caribbean coral reefs (Bayer 1961). Members of the family Gorgoniidae in this region are very diverse and have a symbiosis with zooxanthellae (Sánchez and Wirshing 2005). A particularity of gorgonians is their phenotypic plasticity (Sánchez et al. 2007, Gutierrez-Rodriguez et al. 2008), which enables them to colonize most reef habitats. However,

such variability imposes challenges to delineate species boundaries. Caribbean seafans comprise three species, *G. ventalina, G. flabellum*, and *G. mariae,* all of them include particular scaphoid sclerites (Bayer, 1961). Another characteristic of *Gorgonia* is the reticulate branching forming a mesh-like pattern, which is very dense in all species but *G. mariae*. Some of the growth forms in *G. mariae* are pinnate with occasional fused branches, resembling the genus *Pseudopterogorgia*. Of particular interest, molecular phylogenetic analyses among gorgoniids present different results regarding the position of *G. mariae*, mitochondrial DNA place it with *Pseudopterogorgia* spp. (Sánchez et al. 2003) whereas nuclear DNA (ITS2) with the other *Gorgonia* species (Aguilar and Sánchez 2007). Such incongruence between molecular phylogenies together with the intermediate branching characteristics of *G. mariae*, resembling both *Pseudopterogorgia* and *Gorgonia*, suggest a hybrid origin for this species (see reviews in Seehausen 2004and Mallet 2005). To approach the previous problem, we had two major goals in this study: first was to examine the nature of ITS2 intragenomic variation in seafans and sea whips (*Pseudopterogorgia*), and second to reconstruct preliminary phylogenetic hypothesis of ITS2 intragenomic variants.

Material and Methods

We studied the intragenomic variation in *Gorgonia* spp. and a few species of *Pseudopterogorgia*. We had fresh and museum material from Colombia, Panama, Belize, Puerto Rico and Bahamas. Total DNA was extracted using a CTAB, proteinase K, Phenol – Clorophorm-Isoamyl alcohol extraction method (Coffroth et al. 1992). DNA was resuspended and conserved in TE buffer at –70ºC. DNA quality was checked in agarose (1%) electrophoresis at 80V for 30 min. Using the best DNA extraction quality, primers 5.8s 5`-AGCATGTCTGTCTGAGTGTTGG-3` and 28s 5`-GGGTAATCTTGCCTGATCTGAG-3` designed by Aguilar and Sánchez (2007) were used for the ITS2 amplification, conditions for PCR were as follows: an initial denaturizing step: 2 min at 94ºC, followed by 35 cycles: 30 sec. at 94ºC, 30 sec. at 56.8ºC and 1 min at 72ºC, final extension step: 2 min. at 72ºC. Using 1 unit of Taq polymerase (Invitrogen), 3.5 mM MgCl2, 0.2 mM DNTPs (Biorad Mix), 0.15 uM primers (each) and 4µL of DNA (dilution 1/50) in 20µL as final volume. The amplification was standardized with an efficiency of 95%. PCR reactions were screened in a gel containing 8% polyacrilamide, 1X TAE Buffer and linear urea-formamide denaturing gradient from 45% to 80%. The gels were pre-run at 60ºC and 90V for 30 min, followed by the electrophoresis at 60ºC and 90V for 13 h. Gels were stained with ethidium bromide during 15 min and visualized using a BioRad ChemiDoc system. All reactions were conducted without CG-clamp in the primers. Bands visualized in the DGGE gel were excised using sterilized micropipette tips in the BioRad ChemiDoc system and placed in 1,5 mL tubes with 100µL of sterilized double distilled water. The tubes were incubated in a shaker at room temperature for 24 hours at 150 rpm. Each band extract was collected in a 1,5 mL tube and the DNA was precipitated with 300 µL of cold absolute ethanol, tubes were placed at -20ºC for 24 hours and then centrifuged at 13000 rpm for 30 min, supernatant was discarded and pellet dried and resuspended in 15 µL sterilized double distilled water.

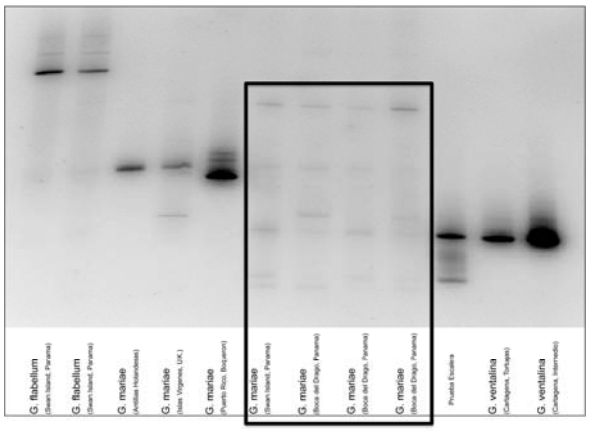

Figure 1: Examples of ITS2 DGGE banding patterns from seafans *Gorgonia* spp. colonies. Inset square: banding patterns of *Gorgonia mariae* from Panama (Bocas del Toro). Linear urea-formamide denaturing gradient of 45% to 80%.

Re-amplification of bands was conducted using PCR as described above except that DNA was used without dilution. Purification of PCR products for sequencing was performed by Exo-Sap method using 1 u of Exonuclease, 0,2 u of Shrimp Alkaline Phosphatase and 2µl SAP buffer 10x per 20 µl in a 0,2 ml tube. Reactions were held at 37ºC for 1 hour and at 80ºC for 15 min. Sequencing reactions were performed with BigDye 3.1 system according to manufactures instructions (Applied Biosystems) and sequenced in a capillary electrophoresis automated sequencer (ABI310). Each sample was sequenced with forward and reverse primers. The consensus sequences were obtained by assembling the two complementary chromatograms in Sequencer 4.7 software.

Secondary structures of all sequences were obtained reconstructing by comparison via Pairwise Alignment (Bioedit) with previously reported structures in octocorals (Aguilar and Sánchez 2007). The sequences were then submitted with a few constrains and restrictions in MFOLD (Zuker 2003) at

a default temperature of 37ºC. The structure chosen was the one with the greater negative free energy but conserving the ring model known for ITS2. The obtained secondary structures were used to correct the alignment using the program 4SALE (Seibel et al. 2006). Phylogenetic analyses included maximum parsimony, maximum likelihood and Bayesian inference (see details in Grajales et al. 2007).

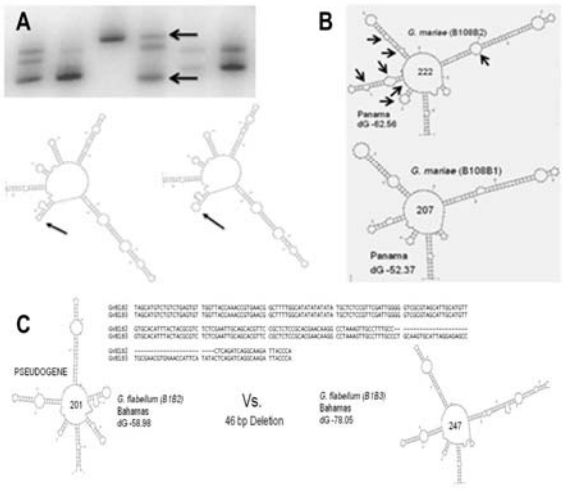

Figure 2: ITS2 predicted secondary structures from the intragenomic variants. A. Two different intragenomic ITS2 variants from an individual colony of *Pseudopterogorgia bipinnata* from Belize, corresponding to one mutation only. B. Two out of five different intragenomic ITS2 variants from an individual colony of *Gorgonia mariae*, including multiple differences. C. Diagnostic DGGE band (left) and a pseudogene (right) lacking most of the proximal helix in *G. flabellum*. Numbers refer to the nucleotide length and arrows point differences among structures.

Results

Gorgonia seafans exhibited a great deal of intragenomic variation as observed with multiple bands in DGGE (e.g., Fig. 1). There were different intragenomic patterns including species with a main "diagnostic" band, or dominant. Some individuals, mainly from *Gorgonia mariae,* did not have a dominant diagnostic band but up to five codominant bands (Fig. 1 inset). The intragenomic variation detected in DGGE gels, after reamplifying and sequencing, included the less amount of variation, a single nucleotide polymorphism (SNP), very common in colonies of *Psedopterogorgia bipinnata* (e.g., Fig. 2A), which was a good indicator that the method was reliable to separate all possible intragenomic variation. In addition, differences among intragenomic variants from the same individual could be highly significant with more than 15 INDELS and substitutions as in the case of *G. mariae* (e.g., Fig. 2B). The extreme case was observed in *G. flabellum* with more than 40 changes between two variants where one of them lacked the proximal helix (Fig. 2C), which should correspond to a pseudogene because it does not have the C2 processing site.

Preliminary phylogenetic analyses among the recovered intragenomic variants from DGGE gels were overall well supported by the three different phylogenetic approaches (Fig. 3). Paralyphyletic intragenomic variants were found in *Gorgonia mariae, P. bipinnata* and *G. flabellum*, although most *P. bipinnata* variants formed a monophyletic group. In contrast, some species such as *G. ventalina*, *P. acerosa,* and *P. rigida* had not variants or exhibited little divergence among intragenomic variants. The only pseudogene found in *G. flabellum* was located completely off *Gorgonia* and *Pseudopterogorgia* species near the outgroup. Although not all the intragenomic variants were successfully recovered from DGGE gels it was clear for *G. mariae* that its intragenomic variants were grouped with both *Pseudopterogorgia* and *Gorgonia* species within well supported nodes (Fig. 3).

Discussion

The study of ITS2 intragenomic variation provides additional compelling evidence for a likely hybrid origin of *Gorgonia mariae*, involving other *Gorgonia* and *Pseudoterogorgia* species as parental sources. Nonetheless, intragenomic variants from other *Gorgonia* and *Psedopterogorgia* species suggest that gene flow among seafans and sea whips might be more common than previously thought. Swarms of interbreeding species, known as a syngameon, have been proposed as a mechanism to quickly promote sympatric speciation via hybridization under adaptive radiation conditions (Seehausen 2004). The suggestive idea of the syngameon has been also proposed for scleractinian corals (Veron 1995, Kenyon 1997) and could explain the great diversity and phenotypic plasticity found in gorgonian corals. The alternative hypothesis about ITS2 intragenomic variation as incomplete lineage sorting of ancestral polymorphisms, might be rejected because ancestral polymorphisms, which should be older, might degenerate and turn into pseudogenes as seen with a corrupted RNA secondary structure. As mentioned before, pseudogenes were found only in *G. flabellum* but were infrequent and easy to detect. Therefore, functional ITS2 copies should be more recent as to prevent complete concerted evolution.

The preliminary phylogenetic hypothesis showed well-supported nodes suggesting multiple origins of *G. mariae* ITS2 intragenomic variants. *G. mariae* could acquire different ITS2 copies through the process known as introgressive hybridization (see review in Mallet 2005). Consequently, unless concerted evolution had occurred, hybridization processes leave

a footprint in the ITS2 generating mosaic copies from diverse parental genomes in *G. mariae*.

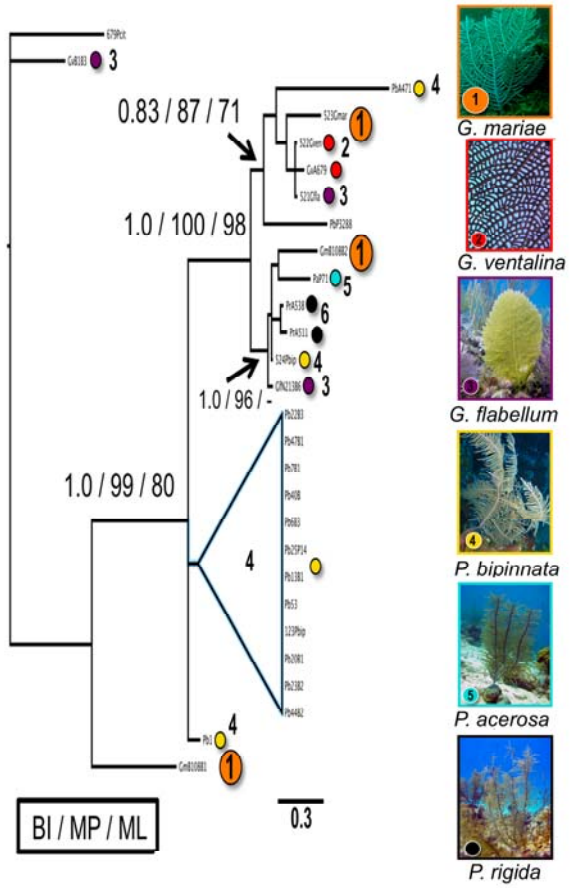

Figure 3: Preliminary phylogenetic results among ITS2 intragenomic variants in Caribbean *Gorgonia* and *Pseudopterogorgia* species. The tree is an optimum Maximum likelihood phylogram showing above node support from Bayesian Inference-BI / Maximum Parsimony-MP / Maximum Likelihood-ML. Scale bar 0.3 substitutions per site. Outgroup: *Pterogorgia citrina*.

Opportunely, scleractinian corals have provided straightforward evidence of introgressive hybridization particularly within species of *Acropora*, which have enlightened on the great potential for introgressive hybridization in sessile marine organisms with broadcast spawning. For instance, Odorico and Miller (1997) found consistent information in *Acropora's* ITSs with a reticulate evolution scenario. Van Oppen et al. (2001) examined diverse nuclear and mitochondrial DNA sequences concluding that paraphylly from most species could be explained by extensive introgressive hybridization and reticulate evolution. Likewise, Marquez et al. (2003) found the presence of ribosomal pseudogenes as a possible consequence of multiple hybridization events. Hybrid origin has also been proposed for soft corals species using ITS (McFadden and Hutchinson 2004). Nonetheless, Vollmer and Palumbi (2004) examined the multiple copies of the Caribbean *Acropora* species and concluded that there is no a proper way to evaluate if the intragenomic shared variation of genes such as ITS1 and ITS2 was the result of incomplete lineage sorting or recent hybridization processes. Yet ancestral polymorphisms should certainly retain more substitutions per site when compared with functional genes and for a ribosomal gene such as ITS2 that means purifying selection acting on secondary structural constraints (Cote and Peculis 2001) or concerted evolution mechanisms acting similarly (Liao et al. 2000, but see Nei and Rooney 2005 and Harpke et al. 2006). It is clear that the ITS2 is not a standard gene for reconstructing phylogenetic hypothesis (e.g., Harris and Crandall 2000) but it seems to retain clues from introgressive hybridization events, which should be examined in detail before reaching a robust conclusion. In conclusion, ITS2 is unfolding a different story of the diversification in octocorals thanks to the aid of techniques such as DGGE (see technological advantages over cloning in: Lajeunesse and Pinzon 2007) and RNA secondary structure prediction, which are techniques strongly recommended for the analysis of this kind of sequences.

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Soft coral biodiversity and distribution in East Africa: Gradients, function and significance

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Abstract. Soft corals (Octocorallia: Alcyonacea) constitute important reef benthos in East Africa, yet relatively little is known of their distributional gradients, function or significance. Integrated results of published surveys manifest interesting gradients in their diversity, abundance and apparent function. Reef disturbance may result in them becoming dominant, eliciting an alternative stable state in some coral communities. While certain tropical taxa attenuate from north to south, others attain their highest abundance at high latitude; the latter appears to be related to their ability to tolerate sedimentation and more swell-driven turbulence. Once established, soft corals appear to be persistent and long-lived. A long-term monitoring study has nevertheless revealed that they appear to be vulnerable to climate change.

Keywords: Soft corals, Alcyonacea, western Indian Ocean, biodiversity gradients

Introduction

Soft corals (Octocorallia: Alyonacea) have been studied on East African reefs at several localities over the last 15 years, including Tanzania (Ofwegen and Benayahu 1992), Mozambique (Benayahu & Schleyer 1996; Benayahu et al. 2002) and South Africa (Benayahu 1993; Benayahu & Schleyer 1995, 1996; Ofwegen and Schleyer 1997; Williams 2000; Williams and Little 2001). These studies have shown that soft corals are abundant in these countries, include descriptions of several new species, and list numerous new zoogeographical records for Tanzania (7°S), Mozambique (12-21°S) and the KwaZulu-Natal coast in South Africa (27.5°S).

The East African coast possesses a full spectrum of reef types, from fringing reefs off the mainland and islands in Mozambique and Tanzania (Hamilton and Brakel 1984; Wells 1988; Obura et al. 2000; Rodrigues et al. 2000) to the high-latitude marginal reefs of southern Africa (Schleyer 2000). Consideration is given here to gradients in the diversity and distribution of the soft corals and their underlying causes, aspects of which have received specific attention (Riegl 1995; Schleyer and Celliers 2003a, b; Schleyer et al. 2008).

The East African coastline under consideration in this study forms the boundary of the western Indian Ocean (WIO) and lies within the influence of the South Equatorial Current (SEC) and its daughter currents. These are generated when the SEC deflects off the northern tip of Madagascar and, in turn, encounters Africa at approximately 10ºS. Lutjeharms (2007) provides the most recent review of their complexity, the deflected currents in question being the East Madagascan, East African and Mozambique Currents. Further complex interactions give rise to the Somali and Agulhas Currents at equatorial and higher southern latitudes respectively.

Materials and Methods

Species lists providing the distributional patterns considered in this paper were derived from material collected in Tanzania; Pemba, Mozambique Island and the Quirimbas, Segundas and Bazaruto Archipelagos in Mozambique; and reefs off Sodwana Bay and Durban in South Africa.

Results and Discussion

Soft coral genera most commonly recorded on East African reefs are listed in Table 1. Less common genera have been omitted for brevity (see publications cited in the introduction for the full record). While some genera proved cosmopolitan, others were found only in the north or south. Most notable was *Cespitularia*, a genus that was locally abundant on reefs recovering from the 1998 El Nino Southern Oscillation-related bleaching in northern Mozambique (MHS pers. obs.; Fig. 1). Its extensive cover in these areas appeared to be opportunistic.

Endemism was high amongst genera in the south, examples listed in Table 1 being *Efflatounaria*, *Eleutherobia* and *Leptophyton*, endemic species of which were narrowly restricted to the South African east coast. This is probably attributable to the transitional nature, from tropical to temperate, of the sea off this coast.

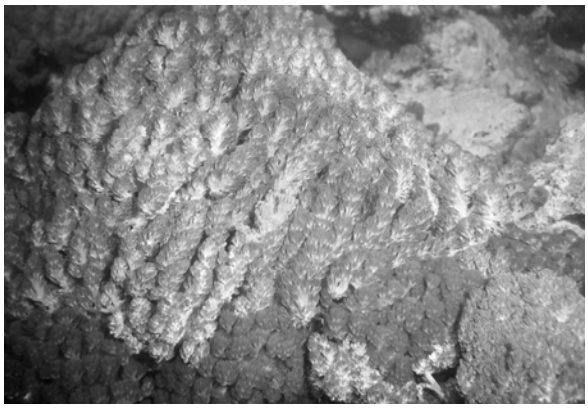

Figure 1. Bleached reef overgrown by extensive colonies of *Cespitularia*.

Table 1. Soft coral genera commonly recorded on East African reefs. TZ = Tanzania; MZ = Mozambique; ZA = South Africa. Grey shading denotes presence of genera and numerals the species, where known.

	TZ	N MZ	S MZ	ZA
FAMILY ALCYONIIDAE				
Cladiella Gray, 1869	5	8	3	3
Eleutherobia Pütter, 1900				2
Klyxum Alderslade, 2000	1			
Lobophytum Marenzeller, 1886	4	2	3	5
Rhytisma Alderslade, 2000		1		
Sarcophyton Lesson, 1834	9	3	5	6
Sinularia May, 1898	18	20	12	15
FAMILY XENIIDAE				
Anthelia Lamarck, 1816		1	1	1
Cespitularia M.-Edws. & Haime, 1850	1	3		
Efflatounaria Gohar, 1934				1
Heteroxenia Kölliker, 1874	2	1		1
Ovabunda Alderslade, 2001				
Sansibia Alderslade, 2000			1	1
Sympodium Ehrenberg, 1834				1
Xenia Lamarck, 1816		1	1	2
FAMILY NEPHTHEIDAE				
Dendronephthya Kükenthal, 1905				
Leptophyton Ofwegen & Schleyer, 1997				1
Lemnalia Gray, 1868	4	3		
Litophyton Forskål, 1775	1			
Stereonephthya Kükenthal, 1905				

Community data, when subjected to similarity analysis (Schleyer et al. in prep), revealed a gradient in abundance of soft corals, relative to the hard, from north to south (Table 2). The latter are dominant in the north but Alcyonacea become more successful in the south.

Table 2. Relative abundance (% cover) of soft and hard corals on East African reefs. TZ = Tanzania; MZ = Mozambique; ZA = South Africa.

Group	N MZ & STZ	S MZ	ZA
N (reefs)	7	3	8
Alcyonacea	6.6	18.7	31.3
Scleractinia	46.6	23.6	25.6

Fabricius (1995) described the slow-growing tenacity of the family Alcyoniidae, in contrast to faster-growing "fugitives" such as the Xeniidae and Nephtheidae. While the opportunistic nature of *Cespitularia* in East Africa has already been mentioned, *Lemnalia* and *Litophyton* were also evident in the north. Persistent alcyoniids, on the other hand, are notably abundant on South African reefs (Schleyer 2000; Schleyer and Celliers 2005; Celliers and Schleyer 2007; Schleyer et al. 2008) where *Sinularia* and *Lobophytum* form extensive carpets, particularly on surge-swept reef crests. This appears attributable to the frequency of large storm waves in the south (Fig. 2).

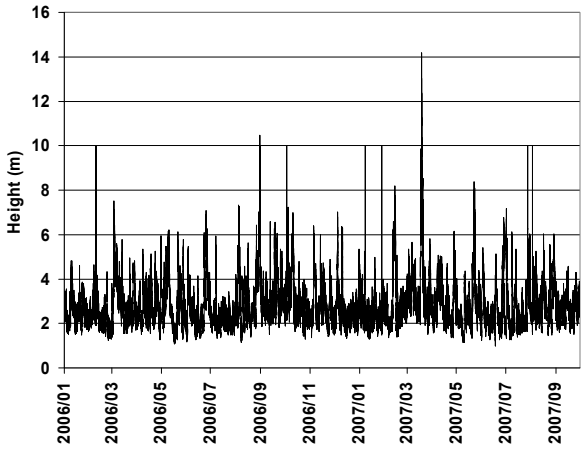

Figure 2. Maximum wave heights (m) recorded between January 2004 and December 2006, 100 km south of the South African coral reefs, portraying the frequency of storm waves in the area.
(Data courtesy of National Ports Authority – Richards Bay)

Persistent alcyonids gained a further competitive advantage during a spot outbreak of crown-of-thorns starfish (*Acanthaster planci*) where infested reef suffered a reduction in hard corals (Celliers and Schleyer 2006, 2007). Persistent soft corals became dominant in these areas, suggesting that their long-

term dominance causes the coral community to enter an alternative stable state.

High turbulence in the south mobilises sediment and a differential abundance of sediment-tolerant soft corals has been recorded on reef tops relative to the reef-sediment interface in South African coral communities (Table 3; Schleyer and Celliers 2003a). Their disposal of sediment is dependent on the turbulence that initially deposits it (Riegl 1995; Schleyer and Celliers 2003a).

Table 3. Relative abundance (% cover) of soft and hard corals in South African coral communities on reef tops relative to the reef-sediment interface (from Schleyer & Celliers 2003a).

	Reef-sediment interface	Reef tops
Alcyonacea	34.6 ± 3.8	31.3
Scleractinia	14.2 ± 4.0	25.6

Factors limiting coral reef development have been assessed by Kleypas et al. (1999), light and aragonite saturation being deemed constraining at high latitudes in the WIO. The resultant reduction of scleractinian competition would further account for the greater success of Alcyonacea in the south. The effects of climate change are anticipated to influence this (Schleyer and Celliers 2003b) and monitoring has revealed that the Alcyonacea have diminished in favour of the hard (Figure 3; Schleyer et al. 2008).

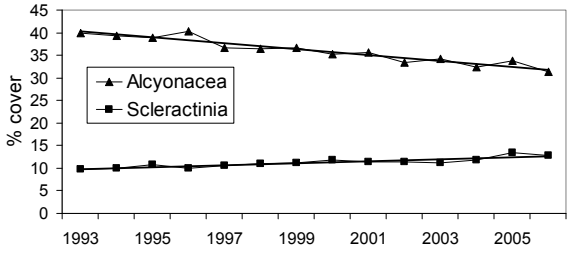

Figure 3. Overall changes in soft and hard coral cover measured at a long-term monitoring site in KwaZulu-Natal 1993-2006.

In conclusion, a number of parameters influence soft coral distribution on the East African coast. Some genera are limited to tropical or more temperate latitudes, with greater endemism at higher latitudes. Opportunism enables fast-growing pioneers to recolonise reef space in the tropics or, conversely, persistent slow-growers to attain dominance in the south. Reduced competition from Scleractinia due to climatic and environmental factors also facilitates the latter. Their proliferation is further promoted by the prevalence of heavy seas at higher latitudes as they are well-adapted to cope with the concomitant turbulence and sedimentation.

Acknowledgments

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Session number 26

Recent Sightings of Longsnout Seahorse, *Hippocampus reidi* in the marine environment of St. Kitts, Lesser Antilles

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Abstract. Only two species of seahorses occur in the Caribbean: the Longsnout, *Hippocampus reidi,* and Lined Seahorse, *Hippocampus erectus*. Between 2007-8, several *H. reidi* specimens were repeatedly sighted at different shallow reef dive sites at St. Kitts. The reefs are mixed habitat with corals, sea grass beds, and sponges. Previous Longsnout seahorse sightings dating back to the early 90s have been inconsistent and restricted to only one of the sites. The recent frequent sightings and identification of several new reef sites could indicate an increase in the local seahorse population size. Further studies are needed to estimate the local seahorse population size and habitat characteristics.

Key words: seahorse population, Caribbean, *Hippocampus reidi, Syngnathus caribbaeus*, pipefish

Introduction

Seahorses (genus *Hippocampus*, family Syngnathidae) are distributed circumglobally in the marine environment. Despite their wide distribution, their life history characteristics (i.e. sparse and patchy distribution, low mobility/site fidelity, low fecundity and, lengthy parental care) make them vulnerable marine species to targeted catch (i.e. traditional Chinese medicine ingredient; aquarium trade) bycatch, and habitat degradation (Foster and Vincent 2004). Since 2004, the entire genus has been added to Appendix II of CITES, thus requiring international trade management by the 166 signatory nations. In the Caribbean, only two species of seahorses occur, namely the Longsnout (*Hippocampus reidi)* and Lined Seahorse, (*Hippocampus erectus)*. The Lined Seahorse is currently listed by the IUCN as a vulnerable species. General information on the abundance of Longsnout seahorse is lacking, and the species has been categorized as data deficient, indicating a need for further studies. *H. reidi* and *H. erectus* are both species used in traditional Chinese medicine.

Material and Methods

St. Kitts (17° 9' N 62° 45' W) is a small Caribbean island of volcanic origin that is part of the Lesser Antilles chain. As part of a large scale local marine ecosystem survey project, data on seahorse/pipefish species, site location, depth, number of specimens, and associated marine habitat were collected during roving diver/snorkel surveys (2006-2008). When feasible, specimens were photo documented. In situ, visual identification of species was based on gross morphological distinct features (ie. color patterns, snout length, body shape, and presence/absence of skin appendages). We are using the taxonomy of Laurie et al. 1999.

Figure 1. Longsnout seahorse at Monkey Shoals

Results

Specific location data, and color patterns for all seahorse sightings are summarized in Table 1. The habitats associated with the sighted seahorses can be classified as mixed habitat with coral gardens, gorgonians, and intermittent sea grass beds. Only one male of 13 cm size and with a large brood pouch was observed. No *H. erectus* specimens were sighted despite the previous confirmed distribution of the

lined seahorse in St. Kitts waters. Longsnout specimens were always associated with holdfast structures such as sea grass, gorgonians, and black corals. In addition to the Longsnout seahorse, individual Caribbean pipefish (*Syngnathus caribbaeus*) specimens were repeatedly sighted in Spring 2008 at South Friars (n=4) and once at Monkey Shoals. The specimens found at South Friars were in shallow water (approx. 6 feet) and occupied shallow, slender rock crevices filled with sand. The specimen found at Monkey Shoals (approx. 25 feet) was found hiding among sea whips.

New Sites	Old Sites
Monkey Shoals *September 2007*: individual: brown *January 2008*: 2 individuals: 1 brown, 1 dark maroon slender male with distinct brood pouch; approx. height 13-17 cm; 1 smaller individual; *May 2008*: individual: brown; *August 2008*: 2 individuals: 1 brown, 1 black; approx. height 13 cm.	**Brimstone Reef** *April 2007*: individual: yellow coloration with white bands along tail; hiding in a sea plume; consistent seahorse sighting over the last several years
Challenger Reef *March 2008*: individual	**Paradise Reef** *June/July 1993*: individual: white blotches along tail
River Taw *September 2007:* individual: very light brown/yellowish	

Table 1: Listing of historic and current seahorse sightings in St. Kitts

Discussion

While Lourie et al. (2004) list *H. erectus* with a distribution for St. Kitts and Nevis, our study demonstrates the presence of *H. reidi* at St. Kitts, but not that of *H. erectus*. Predominant coloration of specimens in St. Kitts was black/brown to orange/maroon, similar to specimens in St. Lucia (Humann and Loach 2004); only one specimen showed yellow coloration with white-brown bands/saddles along tail, similar to a specimen from Little Cayman (Humann and Loach 2004) and Longsnout seahorse specimens identified at Saba (Saba Divers pers. comm.. 2007). Unpublished sightings of the Longsnout seahorse at Brimstone Reef are dating back to the early 1990s but sightings have been inconsistent at this site. The home range of the Longsnout seahorse is small with a reported size of 13.3 m^2 for females and 3.5 m^2 for males (Dauwe 1992), and during breeding season they are thought to show site fidelity. Despite the small home range, only one pair has so far been observed (Table 1) and one individuals had a large brood pouch. The male specimen height was estimated 13-17 cm. Maximum adult height for *H. reidi* is reported at 17.5 cm (Laurie et al. 1999), and 8 cm height at first maturity (Vari 1982). Recorded mean range densities for *H. reidi* in Brazil are 0-51 m^2 and 0–0.006 m^2 (Dias & Ross 2003). Densities in St. Kitts are in the lower density range.

Caribbean pipefish (*Syngnathus caribbaeus*) specimens have previously not been reported from St. Kitts; however their reported Caribbean distribution includes the Greater and Lesser Antilles (Fishbase; Humann and Deloach 2004) where they inhabit weedy or sandy bottoms of shallow inshore waters.

The recent frequent sightings and identification of several new reef sites with Longsnout seahorses could indicate that St. Kitts has a viable and potentially expanding *H. reidi* population. However, St. Kitts, and in particular the Southeast Peninsula, is currently experiencing accelerated development that may impact near-shore marine water quality and resources through construction-related sediment run-off and intensified water tourism (i.e. boating, jet-skiing, snorkeling, diving, marine mammal park). Further studies are urgently needed to better define the current seahorse population size/density in the waters of St. Kitts.

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Cymothoid isopods on coral reef fishes in the near shore marine environment of St. Kitts, Lesser Antilles

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Abstract. Nine species of Cymothoid isopods of the genera *Anilocra* spp. selectively associate with specific West Indian fish species and are location specific. *Anilocra* infestation negatively impacts fish productivity and health. As part of a large scale marine ecosystem survey project of the marine environment (2006-2008), isopod parasitism as a potentially useful marine ecosystem health indicator was monitored during roving diver/snorkel fish species surveys. We report for the first time for St. Kitts the presence of *Anilocra chromis* on brown chromis (*Chromis multilineatus*), but not Blue chromis (*C. cyaneus*), and *Anilocra haemuli* on French grunt (*Haemulon flavolineatum*), Smallmouth grunt (*H. chrysargyum*), Caesar grunt (*H. carbonarium*), and Tomtate grunt (*H. aurolineatum*). Our data supports the previously reported geographic specificity of *Anilocra chromis* infection whereby only Blue or Brown chromis, but never both, are affected within one locality.

Key words: Cymothoid isopods, ectoparasites, coral reef fish, West Indies

Introduction

Cymothoid isopods are permanent ectoparasites of fish. They mostly occur in the near shore coastal marine environment. Cymothoid parasites are protondrous hermaphrodites that attach to the fish surface, mostly around the head region. *Anilocra* infestation is known to impact breeding success, interfere with swimming dynamics, decrease fish size, reduce erythrocyte number, and is known to cause lesions in the host ranging from localized tissue inflammation to underlying bone deformities (Bunkley-Williams and Williams 1998; Adlard and Lester 1995). Nine species of cymothoid isopods (pathogenic parasite) of the genera *Anilocra* spp**.** selectively associate with specific West Indian fish species and are host and site-specific (Bunkley-Williams and Williams 1981).

Material and Methods

St. Kitts (17° 9' N 62° 45' W) is a small Caribbean island of volcanic origin that is part of the Lesser Antilles chain. As part of a large-scale local marine ecosystem survey project, data on cymothoid isopods species, site location, attachment site, number of isopods per fish, and associated marine habitat were collected during roving diver (n=80) /snorkel surveys (n=300) in 2006-2008). When feasible, fish specimens with isopods were photo documented.

Results

In 2007 and 2008, Anilocra chromis on Brown chromis (*Chromis multilineatus*) and *Anilocra haemuli* specimens on French grunts, Smallmouth grunts (*H. chrysargyum*), and Caesar grunts (*H. carbonarium*) were identified during daytime at shallow (< 30 feet) to deep reef sites (> 50 < 100 feet) along the Caribbean side of the island. The associated habitats can be classified as mixed habitat with rubble, coral gardens, gorgonians, and intermittent sea grass beds. Median (min-max) number of isopods observed on coral reef fishes in the marine environment of St. Kitts by survey location is summarized in Table 1.

Anilocra chromis were observed at 93% of the dive sites and at 55% of the snorkel sites. *Anilocra haemuli* on French grunts were observed at 14% of the dive sites and at 77% of the snorkel sites. At none of the dive sites did Caesar and Smallmouth grunts have isopods; however, isopods were present respectively at 22% and 11% of the snorkel sites. Tomtate grunts were affected on 7% of the dive sites and 11% of the snorkel sites. No *Anilocra haemuli* were observed at any of the surveyed sites on other suitable fish hosts such as Coney (*Cephalopholis fulva*), Red hind (*Epinephelus guttatus*), and Graysby (*Cephalopholis cruentatus*). For all fish affected with isopods, the host location of isopods was beneath the eye of host. The observed isopods were of mature size and featured prominently on the face of the hosts. Bilateral isopod association was less common, but not infrequent. We never observed more than one isopod per side.

Location	BC	FG	C	SM	T
Anchors Away (D)	1				
Ballast Bay (S)	9	5 (2-5)	1		
Banana Bay (S)		2 (1-2)			1
Brimstone (D)	10 (2-14)				
Challenger (D)	20				
Cockleshell Beach (S)					
Coconut Reef (D)	3				
Corinthian (D)		1			1
Green Point (D)	3				
Majors Bay (S)	6	(4-9)			
Monkey Shoals (D)	(10-26)	2			
Nags Head (D)	15				
Paradise Reef (D)	8				
Pinney's Beach* (S)	18				
River Taw (D)	(2-12)				
The Rocks (D)	(1-3)				
South Friars (S)		5 (1-20)	2 (1-5)	1 (1-2)	
Shipping Lane (D)	21				
Shitten Bay (S)	30	4			
St. Peter's Reef (D)	3 (1-12)				
Timothy Beach (S)		5 (1-6)			
West Farm (D)	14.5 (1-30)				
Whitehouse Bay (S)	(12-14)	5 (4-10)			

- Nevis, WI.

Table 1: Median (min-max) number of isopods observed on coral reef fishes in the marine environment of St. Kitts by survey location. D indicates dive site; S indicates snorkel site.

Discussion

We report for the first time for St. Kitts the presence of *Anilocra chromis* (Williams and Williams 1981) on brown chromis, but not blue chromis (*Chromis cyaneus*), and *Anilocra haemuli* on French grunts, Smallmouth grunts, Caesar grunts, and Tomtate grunts. Previous reported distribution for *Anilocra chromis* on brown chromis has included the northeastern West Indies, namely Puerto Rico, Mona Island, and the British and US Virgin Islands. Blue chromis have been affected in the Bahamas and Dominican Republic (Williams and Williams 1981). Our data supports the previously reported geographic specificity of *Anilocra chromis* infection whereby only blue or brown chromis but never both are affected within one locality (Williams and Williams 1981; 1982). Isopod attachment as previously described by Bunkley-Williams and Williams (1981) for *Anilocra chromis* and *A. haemuli* is beneath the eye of host (Fig. 1). Affected grunts appeared depressed. We did not measure body size, but no obvious size differences were apparent in affected versus unaffected hosts. Recent work with cardinal fish suggests that *Anilocra* parasitism has significant energetic consequences for the host (Ostlund-Nilsson et al 2005).

Our data indicates subtle geographic differences in cymothoid isopods presence in the marine environment of St. Kitts. *Anilocra chromis* were mostly observed at the dive sites. *Chromis* are preferentially found between 35-80 feet. Thus our results probably reflect their habitat choices. We also observed distinct differences for *Anilocra haemuli* between dive and snorkel sites despite the commonness of grunts at these different depths. In addition, although other fish species (i.e. Coney, Red hind, Graysby) known to be parasitized by *A. haemuli* are present at the snorkel and dive sites, only grunts were affected.

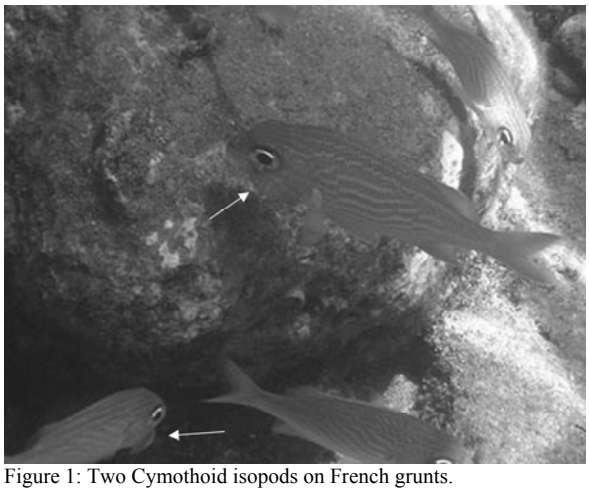

Figure 1: Two Cymothoid isopods on French grunts.

We do not know what environmental and ecological factors (i.e. fish aggregates versus solitary fish, seawater temperature, ocean currents, algae, low light levels, cleaner density) are contributing to the observed geographic differences in cymothoid isopod parasitism. Our preliminary data suggests that there is no difference in presence of cleaner fish between the near shore versus deep sites (Sullivan and Stimmelmayr unpubl. data). Increased fecundity of *Anilocra* spp. in the Mediterranean is linked to warmer water temperature during the summer months (Varvarigos 2003). We did not measure water temperature; however, based on long-term local marine stakeholder observations, seasonal local seawater temperature increases have been occurring earlier and lasting longer. Further studies are needed, including water quality measurements, nutrient content, and temperature monitoring to get a better understanding of the ecological constraints affecting cymothoid isopods in the tropical marine environment. *Anilocra* spp. infestation has a potential to be a useful

marine ecosystem health indicator in a changing environment.

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Habitats and Biodiversity of Ningaloo Reef Lagoon, Western Australia

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Abstract. As part of the CSIRO Wealth from Oceans Ningaloo Collaborative Cluster programme currently underway in Western Australia, this study aims to examine lagoonal habitats and biodiversity within Ningaloo Reef. Key habitat types were identified using information from hyperspectral remote sensing and were used to develop a stratified sampling approach. Two focal areas were selected, based on sanctuary zones within Ningaloo Marine Park: Osprey Bay and Coral Bay in the north and south respectively. A nested quadrat sampling regime was used to attempt to link field-collected data with remotely-sensed data, collected at different scales. Preliminary results confirm that northern sections of Ningaloo Reef differ greatly from the south, with a greater diversity of habitats present in the broader lagoons in the south. Greater areas of coral are found close inshore and across the entire reef at the southern location, compared with the northern section, which has a broad expanse of sand and limestone pavement before grading to corals further offshore (back-reef and reef-crest). These differences in habitat may have implications for the overall biodiversity of the two locations and more broadly along the reef.

Key words: Biodiversity, Ningaloo Reef, habitats, hyperspectral imagery, lagoon.

Introduction

Habitats and biodiversity of lagoonal areas at Ningaloo Reef, Western Australia are currently being studied as part of the CSIRO Wealth from Oceans Ningaloo Collaborative Cluster programme. The aim of the Cluster is to integrate knowledge of reef use and socioeconomics with biodiversity and habitat maps to deliver a management strategy for the Marine Park. This study will deliver high-resolution spatial data on the habitats of the marine and coastal areas of the Ningaloo Marine Park and an understanding of how biophysical characteristics of the ecosystem relate to Ningaloo's biodiversity.

Ningaloo Marine Park extends for some 280 km along the northwest Australian coastline. The unusual circumstances of the poleward-flowing Leeuwin Current, low rainfall and low runoff (resulting in very clear waters), remoteness and strong management regime have resulted in a highly diverse, high-latitude coral reef system in relatively undisturbed condition (Cassata and Collins 2008). The region's low rainfall and low runoff (resulting in very clear waters) makes aerial photography an ideal tool to examine coastal geomorphology and habitat structure. High-resolution hyperspectral aerial photography is being used to provide detailed habitat maps for the entire Ningaloo Marine Park.

The remoteness of this region means that much of the reef is difficult to access. Existing habitat maps have been derived from in-water surveys that have focused on small sections of the reef (Bancroft and Sheridan 2000). The oceanography of Ningaloo Reef is dominated by the southward-flowing Leeuwin Current, which is forced offshore around Point Cloates by the northward-flowing Ningaloo Current (Taylor and Pearce 1999). This effectively divides the Ningaloo Reef into two distinct zones, with the northern zone under stronger influence of the Leeuwin Current than the south, resulting in a potentially higher proportion of tropical species in the northern section of the reef.

Most of the major taxonomic groups have received little or no attention at Ningaloo; biodiversity surveys will especially target the macroalgae, the Cnidaria other than hard corals and the sponges. These groups have had very little work done on them despite the fact that they are critical components of coral reef community structure. This component of the Ningaloo Wealth from Oceans Collaborative Cluster is combining biodiversity surveys and remote sensing

data to develop detailed habitat maps of the entire Ningaloo Marine Park. This paper presents preliminary data from the nested quadrat sampling programme.

Material and Methods

Key habitat types were identified using information from hyperspectral remote sensing and were used to develop a stratified sampling approach. Study areas were also selected to encompass differences caused by current flows along the Ningaloo Reef. The Coral Bay region in the south and Yardie Creek/Osprey Bay in the north have been selected as representative of the northern and southern geographic regions of the reef (Figure 1). Four habitat sub-types were sampled at each location: reef-crest, back-reef, lagoon and inner reef-flat. This paper describes preliminary data from nearshore sampling.

Sampling strategies included monitoring benthic habitat types along transects, nested quadrats to match up with the pixel size obtained by remote sensing, and transects to quantify numbers of key invertebrate species (holothurians, tridacnid clams, and the corallivorous snail, *Drupella*).

The nested quadrat design is intended to provide a link between the remotely sensed data (3.5 m pixel size) and diver-monitored benthic sampling. 9 x 9 m quadrats were marked out underwater and divided into nine 3 x 3 m sub-quadrats (Figure 2) (McDonald 2007). Estimates of benthic cover were obtained for the large quadrat and sub-quadrats using modifications of the categories developed for the Australian Institute of Marine Science long-term benthic monitoring programme (English et al. 2004). A minimum of three nested quadrats were sampled for each sub-region; further sampling is currently underway.

Results

Data presented here include preliminary nested quadrat samples from lagoon areas within Osprey Bay, Yardie Creek and Five Finger lagoon, which is within the Coral Bay region. All sites showed a predominance of sand, macroalgae and rubble as expected; the key difference observed was a significant level of soft coral cover at the Osprey Bay site (Figure 3). The pie charts represent the major benthic categories within the lagoonal areas at the three study sites (mean of 5, 6 and 3 samples at Osprey Bay, Yardie Creek and Five Finger Lagoon respectively). Osprey Lagoon showed a greater proportion of sandy substrate although the diversity of substrates was greater than the other sites. The dominant categories have been highlighted in each chart for clarity.

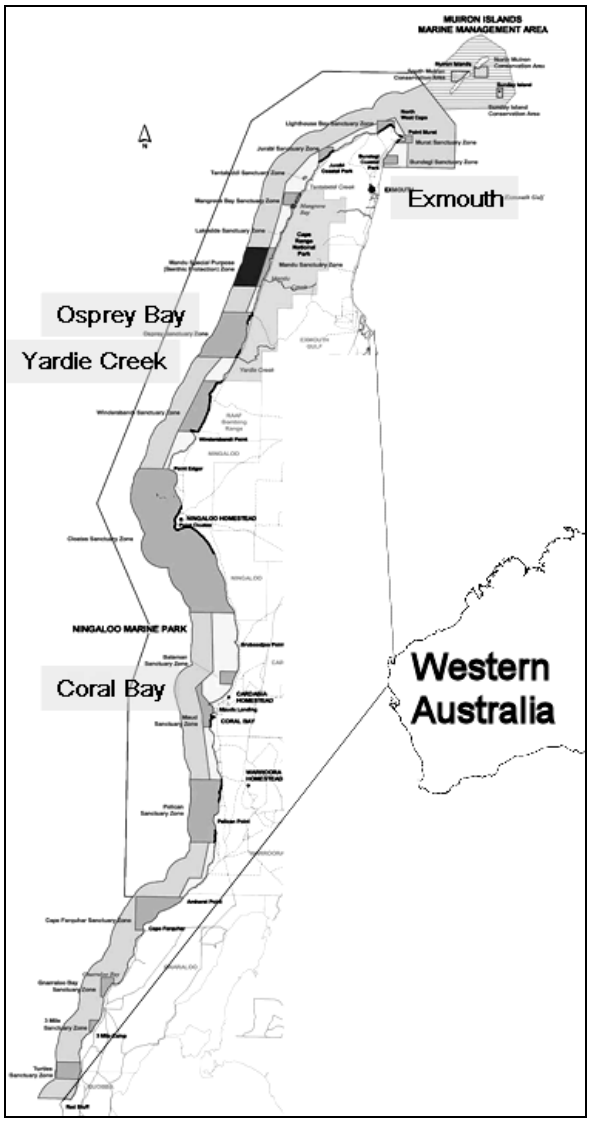

Figure 1: Ningaloo Marine Park in NW Australia. The Park boundary is indicated as State waters (to 3 nautical miles) and Australian Commonwealth waters (to 12 nautical miles). The specific study sites, Coral Bay, Yardie Creek and Osprey Bay are marked.

Discussion

The biodiversity sampling associated with the CSIRO Wealth from Oceans Ningaloo Collaborative Cluster programme has two primary purposes: firstly, to validate the hyperspectral data used to develop detailed habitat maps of the Ningaloo Marine Park; and secondly, to help fill gaps in the biodiversity knowledge of the Ningaloo Reef system. The data collected by the process described in this paper provides excellent validation for the hyperspectral remote sensing programme and good spatial determination of benthic habitat categories. Preliminary habitat classification was used to identify possible field sampling targets and this provides a

powerful mechanism for obtaining both confirmation of the classification process but also the relevance of the benthic categories being used in water. Additional validation sampling has included cross-reef transects, point sampling and diver-towed in-water GPS records of identifiable features.

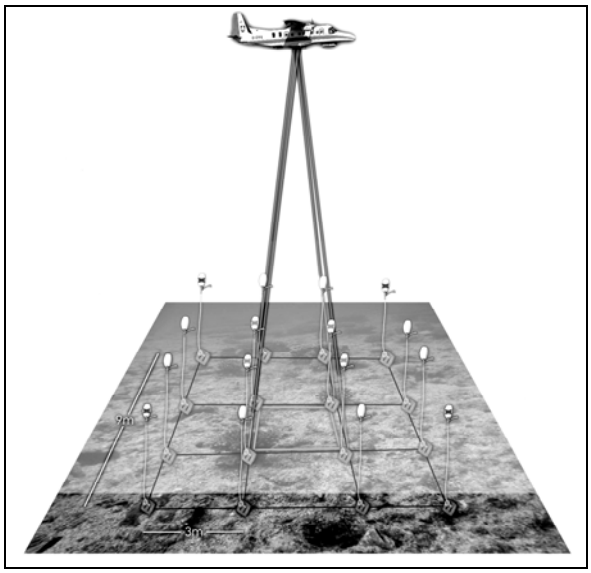

Figure 2: Schematic of nested quadrat design. Large quadrats are 9 x 9 m, sub-divided into nine 3 x 3 m sub-quadrats, approximately matching the aerial photography pixel size of 3.5 m.

Studies of oceanographic processes offshore of Ningaloo Reef indicate an interruption to the flow of the major local currents at Point Cloates, located approximately halfway along the length of the reef. At this point the southward flowing Leeuwin Current is forced offshore by the Ningaloo counter-current, which loops back as an eddy (Taylor and Pearce 1999). This interruption of the warm, nutrient-poor Leeuwin Current has considerable implications for trophic relationships on the reef. One of the preliminary observations that can be made from the data presented in this paper is the difference in major taxonomic and substrate categories identified at the sample sites. While sandy substrates predominate at all three sites (as expected from lagoon samples), there is a significant component of soft corals present in the Osprey Lagoon samples which does not occur further south. Conversely, the southern site is dominated by macroalgae, coralline algae and tabulate *Acropora*. It is anticipated that further samples from other sectors of the reef will shed more light on these differences in taxonomic categories and benthic habitats.

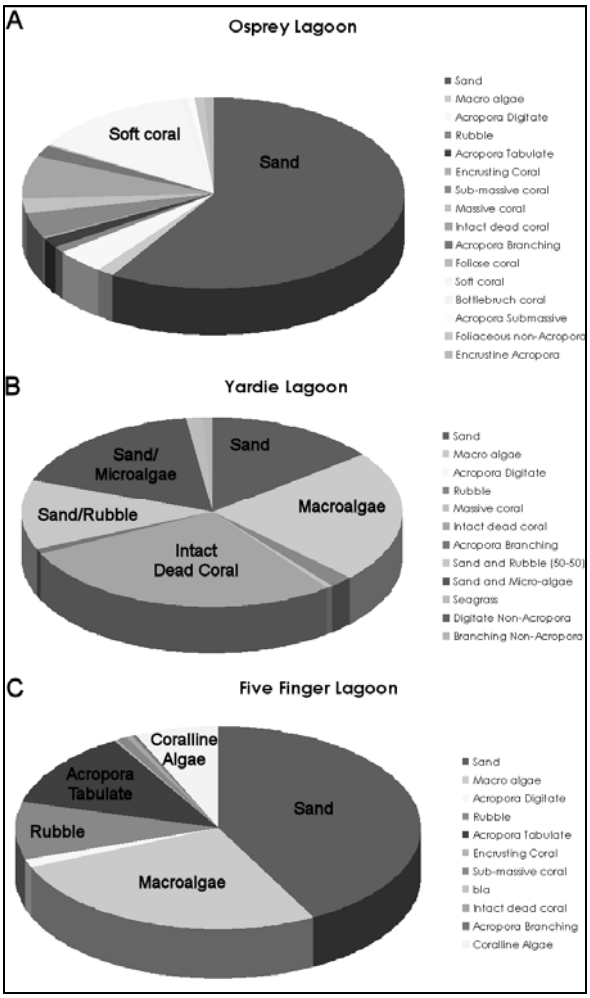

Figure 3: Major benthic categories identified from benthic nested quadrat surveys at A) Osprey Lagoon (n = 5), B) Yardie Creek Lagoon (n = 6) and C) Five Finger Lagoon (n = 3). Dominant categories have been labeled for clarity.

When combined with bathymetric data also derived from the hyperspectral aerial photography it is anticipated the habitat maps generated from this project will provide a framework for studies examining biodiversity patterns and factors influencing the distribution of significant wildlife aggregations in the region. The data generated by this study will be integrated with several other biodiversity studies currently underway in the Ningaloo region and will help to inform management decisions about the Ningaloo Marine Park.

Acknowledgement
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Coral systematics inferred from the gene galaxin: Exploring phylogenetic relationships using a putative determinant of skeletal morphology

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Abstract. Molecular phylogenies and morphological datasets may not entirely agree, and often fail to accurately reconcile many phylogenetic uncertainties. In corals, one way of resolving this disagreement is to complement the existing sets of genetic markers by identifying and analyzing genes directly responsible for generating morphology. Since traditional taxonomy in corals has been dependent almost entirely on their biomineralized skeletal morphology, the use of genes controlling these processes may help identify cases where morphological taxonomy has led to different conclusions about scleractinian evolution. To test this approach, we used the cDNA sequence of the protein galaxin, isolated from the organic matrix of *Galaxea fascicularis*, to amplify a ~363bp DNA fragment from 18 scleractinian species in 8 genera. Translated amino acid sequences revealed minimal within-species sequence variation within *G. fascicularis* collected from 5 different geographic locations. Phylogenetic reconstruction found three well-supported clades, one containing members of the Acroporidae (Complexa), one comprising members of the Oculinidae and Euphyllidae (Complexa), and one comprised of genera from the Siderastreidae, Faviidae and Agariciidae (Complexa and Robusta). These preliminary results illustrate an approach which may help better understand the morphological variation upon which traditional taxonomy has been based.

Key words: coral, systematics, phylogenetics, taxonomy, galaxin, DNA, scleractinia.

Introduction

Uncertainties in scleractinian taxonomy are rooted in the inability to accurately decipher morphological homologies. Formal taxonomic descriptions of scleractinians have utilized a variety of morphological features ranging from septal microstructure (Wells 1956) to corallite and colony morphology (Veron 1995, 2000) to create taxonomic classifications that imply the diversification of the group. Molecular DNA approaches provide an opportunity to independently test these taxonomic hypotheses. Over the last ~12 years, the systematic relationships of scleractinian corals have been challenged by phylogenetic reconstructions based on nuclear and mitochondrial DNA (Romano and Palumbi 1996, Chen et al. 2002, Fukami et al. 2004, 2008). These studies recovered phylogenies that do not agree with many of the traditional taxonomic assignments within the Scleractinia, particularly at the subordinal and family levels.

At least two major themes have arisen concerning the evolution of the scleractinia based on DNA evidence. The first theme asserts the scleractinian lineage is comprised of two major clades – the "robust" and "complex" corals (Romano and Palumbi 1996, 2000, Chen et al. 2003). The "robust" corals consist of heavily calcified corals as a result of a dense construction of their corallite walls (e.g. septothecal or parathecal). In contrast, the "complex" corals contain many corals that build more lightly calcified skeletons due to a relatively porous skeletal assembly of their corallite walls (e.g. synapticulothecal). A second theme states that there is pervasive morphological convergence of many Atlantic and Pacific scleractinian genera (Fukami et al. 2004, 2008). Many Atlantic genera are more closely related to each other than they are to their Pacific congeners and *vice versa*. This implies that many Atlantic and Pacific lineages each experienced their own intra-basin radiations and converged on similar morphological features, detected by examination of their corallite wall architecture.

Other studies have narrowed the link between molecular phylogenies and the biomineralization patterns of corallite microstructures in order to determine homology among taxonomically questionable groups. Benzoni et al. (2007) examined septal micro-features of the centers of calcification of *Psammocora* spp. of debatable taxonomic affinity. Cuif et al. (2003) demonstrated that the growing

edges and numerous granules and spines related to septal growth, once considered unimportant ornaments, are important features of the initial skeletal framework and contain valuable phylogenetic information.

All of these studies assume that the molecular markers used can serve as accurate proxies for the evolution of their taxonomic characters, and can thus be tied to morphological changes in those taxa. In many cases, this assumption has been validated and accurate corroborations between molecular phylogenies and coral morphologies have elucidated many novel evolutionary relationships (e.g. Romano and Palumbi 1996, Fukami et al 2004, 2008). However, many systematic relationships still remain unresolved because of the inability of the molecular markers used to unequivocally resolve all of the systematic uncertainties within the Scleractinia. An approach to solving this problem is to augment the current regime of scleractinian molecular markers by identifying and analyzing genes directly responsible for generating morphology. Unlike most organisms (whose taxonomy is based on many phenotypic characteristics that collectively can not be pinned to a single genetic function), coral taxonomy is almost exclusively based on their skeletal (calcium carbonate) morphology that is genetically controlled by the biomineralizing machinery of their genomes. This characteristic offers the possibility to utilize these molecular "drivers" (genes responsible for calcification/morphology) in concert with existing molecular markers in order to potentially identify areas where morphological taxonomy may have led to different conclusions concerning scleractinian evolution.

This study represents an initial look at the utility of the gene galaxin, a region that encodes for a protein isolated from the organic matrix (a consortium of proteins that forms the scaffolding for biomineralized structures) of the coral *Galaxea fascicularis* (Fukuda et al. 2003), for phylogenetic reconstruction.

Material and Methods

Samples were collected by coring a 1cm x 1cm fragment from individual coral colonies. Tissue and skeletal material were stored in either 95% EtOH or saline DMSO. Total genomic DNA was extracted by established protocols (see Rowan and Powers 1991). Primers Gal16(F) 5'-GGAGCTACCCAGTTATGCTG-3' and Gal13(R) 5'-TATGCTAGCCCAGCACAGGAT-3' were designed from the published cDNA sequence of the gene galaxin (Fukuda et al. 2003) and were used to amplify all *Galaxea* spp., *Euphyllia* spp., *Diploria labyrithiformis*, *Pachyseris speciosa*, and *Acorpora cervicornis* samples. Primer Gal13mc(R) 5'-TATGCAAGTCCTGCGCAAGAT-3', designed from the galaxin sequence of *Montipora capitata* (Accesion # EU022118) was used with primer Gal16(F) (see above) to amplify all *Montipora* spp., *Siderastrea* spp., and *Solenastrea bournoni* (Table 1). PCR (polymerase chain reaction) was conducted using Promega GoTaq® DNA polymerase and provided buffers. Thermocycler reaction parameters were: initial denaturing step at 94°C for 3 min., followed by at total of 35 rounds of 94°C for 1min., 50°C for 1min., and 74°C for 1min. A final extension step of 72°C for 7min completed the reaction. PCR amplifications were confirmed by running products on 2% agarose gels. All successful amplifications were directly sequenced on an ABI 3730 sequencer and forward and reverse sequence contigs were edited with the software ContigExpress in Vector NTI Advance™ 10 (www.Invitrogen.com).

Primer Pair	Species (with ID)	Accesion #
Gal 16(F)/ Gal 13(R)	*Galaxea fascicularis* (AS301)	TBA
	Galaxea fascicularis (MLD197)	TBA
	Galaxea fascicularis (WA270)	TBA
	Galaxea fascicularis (MA142)	TBA
	Galaxea fascicularis (JP581)	TBA
	Galaxea astreata (GA33)	TBA
	Euphyllia ancora (JP163)	TBA
	Euphyllia glabrescens (JP603)	TBA
	Euphyllia yaeyamaensis (JP655)	TBA
	Diploria labyrinthiformis (B56)	TBA
	Pachyseris speciosa (MLD1)	TBA
	Acropora cervicornis (B38)	TBA
Gal 16(F)/ Gal 13mc(R)	*Montipora faveolata* (JP426)	TBA
	Montipora mollis (WA219)	TBA
	Montipora caliculata (AS237)	TBA
	Montipora monasteriata (AS216)	TBA
	Montipora grisea (AS219)	TBA
	Montipora verrucosa (JP 447)	TBA
	Siderastrea siderea (KL101)	TBA
	Siderastrea radians (KL55)	TBA
	Solenastrea bournoni (KL86)	TBA

Table 1: Coral species amplified, and GenBank Accesion #'s, with custom oligonucleotide markers designed from galaxin cDNA sequences of *G. fascicularis* (Fukuda et al. 2003) and *M. capitata* (Accesion # EU022118). ID codes contain geographic location and collection number. AS=American Samoa, MLD=Maldives, WA=Western Australia, MA=Mauritius, JP=Japan, GA=Gulf of Aqaba, B=Belize, KL=Key Largo.

DNA sequences were translated into amino acid sequences using GeneDoc (Nicholas and Nicholas 1997) and aligned using MUSCLE (Multiple Sequence Comparison by Log-Expectation) (Edgar 2004). ProtTest (Abascal et al. 2005) was used to determine the model of amino acid substitution that best fit the alignment. Phylogenetic reconstruction was performed using Bayesian Maximum Likelihood (MB) in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). Tree space was explored using 4 Markov chains for 1,000,000 generations with trees sampled every 100 generations. Posterior probabilities were calculated by setting the "burn-in" to 2500 (25% of

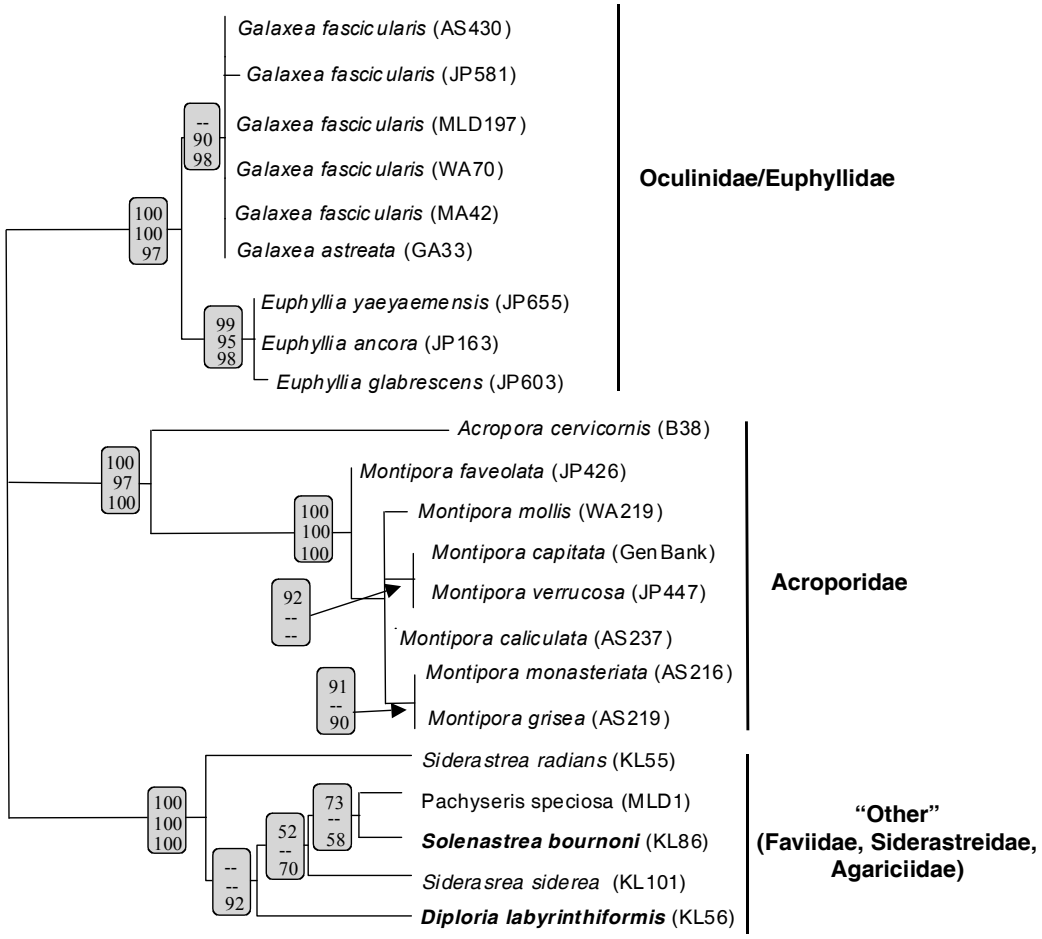

Figure 1: A cladogram depicting relationships recovered by Bayesian Maximum Likelihood (MB), Maximum Parsimony (MP), and Minimum Evolution (ME). Posterior probabilities (MB) and bootstrap values for MP and ME >50 for each node are shown in boxes from top to bottom, respectively. Vertical lines on right highlight clades that correspond to familial groups recovered (Oculinidae/Euphyllidae and Acroporidae) or where relationships were uncertain ("other"). "Robust" corals are in bold. "Complex" corals are in normal font. "--" indicates node <50 or not following branching topology for that method.

sampled trees), and values were generated from the remaining 7500 trees. Maximum Parsimony (MP), with branch swapping set to TBR (tree bisection-reconnection) and starting trees gained by stepwise addition (random), and Minimum Evolution (ME) (default parameters for amino acid sequences) were performed using PAUP* v4.0b10 (Swofford 2002). 1000 bootstrap replicates were performed for each method.

An appropriate outgroup to root the phylogenetic trees was not available, as the gene galaxin has not been found outside the Scleractinia. As a result, all trees were rooted with midpoint rooting in PAUP*. MrBayes does not allow for midpoint rooting, so no root was used.

Results

The sequenced PCR fragments, after editing, were 363bp in length except for the *Monitpora* spp. and *Acropora cervicornis*, which were 357bp. Translated amino acid sequences were, thus, 121 and 119 amino acids in length. Two indels, of one amino acid each, were located at the 9th and 37th positions in the alignment (data not shown). ProtTest found the model Dayhoff + G to best fit the alignment with both Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) frameworks.

All three methods of phylogenetic reconstruction found three major clades with high statistical support – *Galaxea* spp.-*Euphyllia* spp., *Montipora* spp.-*A. cervicornis*, and *Siderastrea* spp.-*Pachyseris speciosa*-*Solenastrea bournoni*-*Diploria labyrinthiformis* (Fig. 1). The phylogenetic relationship among them was unresolved. The *Montipora* spp.-*A. cervicornis* clade corresponds to generic memberships within the family Acroporidae. The *Galaxea* spp.-*Euphyllia* spp. clade unites genera from the families Oculinidae and Euphyllidae. And a third clade joins species from four genera and three families (Faviidae-Siderastreidae-Agariciidae).

The *G. fascicularis* from five different geographic locations (Table 1) were invariant in their amino acid sequences except for one change (threonine to serine) in the Japan individual at position 64 in the alignment (data not shown). Among congeners, the relationships among the three species of *Euphyllia* were unresolved. The *Montipora* spp. did, however, show some structuring, although statistical support was inconsistent among all phylogenetic reconstruction methods. For example, *M. capitata* and *M. verrucosa* grouped as supported sister species with MB (92) only, and *M. monasteriata* and *M. grisea* fromed a clade supported by MB (91) and ME (90) (Fig. 1).

The "Faviidae-Siderastreidae-Agariciidae" clade contained unexpected relationships, most of which were not supported by high bootstraps values or posterior probabilities (Fig. 1). For example, *S. radians* and *S. siderea,* two well-known morphological sister species, did not group monophyletically.

Discussion

The organic matrix of corals is a consortium of proteins that is intimately associated with the calcification process in many organisms including corals (Simkiss and Wilbur 1989). Cuif and Dauphin (2005) describe the calcification procedure in corals as a biochemically driven process where genetically controlled coral fibers, of which sulfated acidic proteoglycans are likely to play a significant role, guide calcification in repeated cycles. Similarly, Meibom et al. (2008) state that the morphology of the coral skeleton is related to the genetically defined positions and distributions of their centers of calcification. It is, therefore, plausible to assume that much of the evolutionary history of the coral skeleton, including convergences, may be found in the genes involved in the biomineralization process of the coral skeleton. It is still unknown, however, to what degree (or if at all) organic matrix proteins, including galaxin, play in that process.

Here, we use only ~40% of the organic matrix protein sequence of galaxin across several coral taxa. We utilized translated amino acid sequences in order to examine any variation in the protein sequence that may account for morphological differences in coral skeletons. Our phylogenetic reconstruction employed only a small set of scleractinian species due to the inability to successfully amplify galaxin across divergent taxonomic groups. Our tree, however, recovered many traditional taxonomic groupings based on morphology as well as groupings based on studies using other DNA markers. For example, the *Montipora* spp.-*A. cervicornis* clade recovered corroborates the well-established morphological relationship between these two genera (e.g. Wells 1956), both of which are placed within the family Acroporidae. This relationship has also been verified by molecular phylogenies using both nuclear and mitochondrial DNA (Romano and Palumbi 1996, Chen et al. 2002, Fukami et al. 2008). Although not well supported by all of the reconstruction methodologies used, the pairing of four *Montipora* spp. (*M. capitata* with *M. verrucosa* and *M. monasteriata* with *M. grisea*) corroborates morphological similarities among these species. For example, both *M. capitata* and *M. verrucosa* share large coenosteum tuberculae forming verrucae and *M. monasteriata* and *M. grisea* approach each other morphologically with their coenosteum tuberculae and thecal papillae (Veron 2000).

The close relationship between the *Galaxea* spp. and *Euphyllia* spp. recovered is similar to that found by Fukami et al. (2008). Although *Galaxea* is in the family Oculinidae (Veron 2000), it likely shares a closer phylogenetic history with members of the family Euphyllidae (Fukami et al. 2008).

The "Faviidae-Siderastreidae-Agariciidae" clade contained unexpected groupings. The polyphyly of *S. radians* and *S. siderea* is an example. Given the poor statistical support for the branching topology within this clade, however, it is likely that insufficient sampling and/or the lack of phylogenetic information available at this locus resulted in the uncertain relationships found.

All of the taxa used in this analysis are considered "complex" corals except two species, *S. bournoni* and *D. labyrinthiformis* (see Romano and Palumbi 1996 and Fukami et al. 2008). Trends concerning the evolution of the "complex" and "robust' corals were, therefore, not possible. Our "Faviidae-Siderastreidae-Agariciidae" clade grouped two "robust" corals, *S. bournoni* and *D. labyrinthiformis*, with three "complex" coral species (Fig 1). The relationship of *P. speciosa* to these "robust" corals is inconsistent with Fukami et al. (2008). They recovered *P. speciosa* as a sister taxon to a clade containing *Galaxea* and *Euphyllia* spp. However, *Siderastrea* branches divergently among the "complex" corals (Fukami et al. 2008), and it is, therefore, likely that both *S. bournoni* and *D. labyrinthiformis* would share a closer phylogenetic affinity to the *Siderastrea* than to the other taxonomic groups in our phylogeny.

This study represents the first use of an organic matrix protein to reconstruct phylogenetic relationships among corals. There is still much that is not known about the gene galaxin or the biomineralization process in corals. For example, how many other genes are involved in the calcification process? And how exactly do organic matrix proteins influence morphology? The gene

galaxin is likely part of a gene family, and there are at least two galaxin-type genes indentified (D. Miller, unpub. data). It is still uncertain how these genes, and the proteins they encode, affect skeletal morphology and what they may reveal about the evolutionary history of the entire scleractinian lineage. Future work will involve complementing organic matrix loci with other established molecular markers. A complementary approach should help unravel the uncertainties of scleractinian evolution and possibly highlight areas where morphological taxonomy and molecular phylogenies have been confounding and/or add corroborative support to established relationships.

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Need for a more integrative approach to scleractinian taxonomy

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Abstract. The history of scleractinian taxonomy is divided into six periods: the original, purely typological, was based on scarce coralla material; the second, starting at the end of nineteenth century, originated with visiting natural habitat and established variability; the third commenced in the 1930s with detailed skeletal study; the fourth began in the 1950s with scuba access to coral habitat; the fifth began in the 1980s following fundamental discoveries in life history and molecular genetics; and the sixth started in 1995, when data from molecular genetics opened a new avenue for scleractinian megataxonomy, contradicting conventional gross-morphology taxonomy but resulting congruent with skeletal histology and ornamentation. Currently, there are four sources of scleractinian taxonomic information: morphology, paleobiology, developmental biology and molecular genetics. Taxonomy is important for understanding scleractinian biodiversity and reef conservation. However, the taxonomy is fragmented and the nomenclature tangled. The e-dimension benefits have not yet been realized and the New Taxonomy not yet arrived for scleractinians. Vision and teamwork are needed for a more integrative taxonomy. The Atlantic Scleractinia Initiative seeks to address the following points: massive sampling, study of the mesophotic habitat, collection access, analysis of phenotypic diversity, life history, geological history, molecular genetics, cyberinfrastructure and education of specialists.

Key words: Scleractinia, taxonomy, microstructure, biodiversity, reefs.

Introduction

For a long time, the taxonomy of scleractinians was based only on macromorphologic skeleton characters. This changed fundamentally in the second half of the last century, and even more during the last two dozen years, following a series of important discoveries related to skeletal ornamentation and histological microstructure, paleobiology, ecology, roll in the reef ecosystem, life history and molecular genetics. With new knowledge, the taxonomy became very complex, and it now plays a crucial role in the understanding of scleractinians, their biodiversity and importance for the fragile reef system.

This note provides a brief historical review of scleractinian taxonomy and an analysis of its current state, and concludes with recommendations for a more integrative approach.

History

The history of scleractinian taxonomy can be divided into six periods which demonstrate its accelerated development.

First period

Originally, studies were based on scarce coralla material found on beaches or during fishing activities, and the taxonomy was *purely typological*.

Second period

At the end of the nineteenth century, investigators began visiting the natural coral habitat. Field observations and systematized sampling demonstrated the presence of *scleractinian variability*. The forma category was introduced as a taxonomic subdivision of the species (Quelch 1886; Vaughan 1901, 1907). Transplantations first demonstrated induced change in coral growth form (Vaughan 1911). During the first half of the twentieth century, these corals did not attract much attention from zoologists. Two American paleontologists synthesized the knowledge on Scleractinia (Vaughan and Wells 1943; Wells 1956).

Third period

In late 1930s, in France, another paleontologist commenced a revision of the classical fossil coral collections, paying special attention to skeletal ornamentation and histological microstructure (Alloiteau 1952, 1957), and put together a team of specialists on scleractinians of different geological periods. The ornamentation of the radial elements was considered to reflect their histological structure and was used in the scleractinian systematic. The taxonomy was based on *more skeletal characters* than previously. Unfortunately, this improved taxonomic methodology was not applied for long outside the

disciples of the Parisian school, which may have been due to language barriers.

Fourth period

Starting in the 1950s, scuba made the *coral habitat accessible* for investigators. Pioneering work (in order of execution) in Jamaica (Goreau 1959, Goreau and Wells 1967; Wells 1973), the South Pacific (Chevalier 1968, 1971, 1975), Madagascar (Pichon 1964), the Red Sea (Scheer, 1964; Loya and Slobodkin 1971), Cuba (Zlatarski 1982) and Australia (Veron and Pichon 1976, 1980, 1982; Veron and Wallace 1984; Veron et al. 1977) established an extraordinary variability of the skeletal morphology on a global scale and shed light on the role of Scleractinia in the reef-building process. These findings coincided with the results of massive paleontological sampling of scleractinians from different geological periods, from their appearance in the Triassic to the present. The global spatial and temporal variability translated into serious taxonomic difficulties for species identification, which is basic for reef study. A reevaluation of the scleractinian taxonomy was needed (Zlatarski 2007). The last synthesis on scleractinians (Chevalier 1987) presented the enormous mass of information collected to date and the dilemmas of this period.

Fifth period

Since the 1980s, a series of *fundamental discoveries in life history and molecular genetics* broadened very quickly the existing scleractinian knowledge in uncharted aspects: long generation times and frequent propagation fragmentation (Potts 1984), simultaneous multispecific spawning (Harrison et al. 1984; Oliver and Willis 1987), reticulate concept for coral speciation (Veron 1995), lateral gene transfer or hybridization (Willis et al. 1997), astonishing growth forms in aquaria (Carlson 1999), coral symbiosis (Hoegh-Guldberg 1999), coral health and the role of microorganisms in relation to it (Rosenberg and Loya 2004; Reshef et al. 2006), and the notion of coral holobiont (Rosenberg et al. 2007). Research interest in scleractinians ceased to be focused exclusively on morphology. Instead it expanded widely in numerous new directions (Zlatarski 2007) and became an important and integral part of reef investigations. The time had come to reorient scleractinian research and for a basic change in the taxonomy.

Sixth period

So much has been learned over the past dozen years, as molecular genetics opened a new avenue for scleractinian megataxonomy (Chen et al. 1995; Veron et al. 1996; Romano and Palumbi 1996; Romano 1996; Romano and Cairns 2000). Strikingly, due to the findings of homoplasious gross-morphology characters, the results were non-congruent with the conventional taxonomy, based on macromorphologic characters. Most traditional supra-families and some families and genera were recognized not to be monophyletic units and this led to a taxonomic impasse. Not surprisingly, the solution came from the previously-introduced (during the third period) but neglected methodology of more detailed morphological study, and from rediscovering the septocentric scleractinian taxonomy (Stolarski and Roniewicz 2001; Stolarski and Russo 2002; Fukami et al. 1994; Cuif et al. 2003; Stolarski and Vertino 2007). Importantly, it turned out that *the molecular information is congruent with data of skeletal histological structure and ornamentation*.

Today

The current situation

Today there are four sources of scleractinian taxonomic information: morphology, paleobiology, developmental biology and molecular genetics. Use of all four is necessary for optimal success. Taxonomic decisions based on only a portion of the available sources are neither satisfactory nor justified. Taxonomic projects are no longer possible for the solo investigator and require teams and networks.

Fundamental biological and taxonomic issues such as species definition and the process of speciation continue to be the subject of discussion. The question of how much reproductive isolation is required between "good" species remains open (Abbott et al. 2008) and applies frequently to scleractinians. Also for these corals, phenotypic plasticity (environment-induced changes in morphology) is usually not distinguished from intraspecific genetic variation (polymorphism) (Todd 2008). The latter offers a probable explanation for the existence of some instances of morphogenesis extravaganza. For example, in environments with calm waters, a lack of hard substratum and the presence of very fine sediments, it looks like mutational accidents were not eliminated by selection, and evolution led to exuberant endemic colony astogeny. In the same train of thought, the endemic morphological richness of the Lower Cretaceous scleractinians of Diplocteniopsidae (Zlatarski 1968) is reminiscent of speculations regarding the origin of the rich Burgess shale fauna (Mayr 2001).

The taxonomy is fragmented in a number of ways and for a variety of reasons. First, some taxonomic works are purely descriptive while others have a predominantly phylogenetic orientation. Neither extreme benefits taxonomic efforts. Second, researchers rely on literature published during a very long period, the last 250 years, and it is not always

easy to access this literature. Third, the publications are in various languages, requiring a certain linguistic ability. Fourth, investigators need to study the existing collections, but sometimes these are not available or access to them is restricted. Fifth, the research facilities are frequently far from the natural habitat. In addition to all of these obstacles, taxonomists need to untangle a multitude of nomenclatural challenges.

These impediments pose serious challenges and require a reorganization of the taxonomic work utilizing all available sources as well as a mastery of professional skills, nomenclature and ethics.

The e-dimension

In the face of our current taxonomic difficulties and the gathering clouds over coral reef survival, there is a silver lining in the potential offered up by electronic communication. It has quite aptly been stated that taxonomy is made for the Internet (Godfray 2002). The cyberinfrastructure offers on a global scale not only ways for accessible and efficient communication between investigators, but it is also diminishing the disadvantages caused by the fragmentary character of taxonomy. Taxonomic work may benefit from the online availability of rare publications; digitized collections and virtual usage of types and specimens; storage of rich descriptive data and illustrations; and reduction of the distance between researchers and major institutions. The e-dimension is open to a wide audience, including "purist" taxonomists, specialists dedicated to reef conservation, educators and laymen. It permits taxonomy to be vested with authority and at the same time not to be authoritarian. The advantages of the e-dimension for scleractinian taxonomy are yet to be realized.

The New Taxonomy

The 2005 international Biennial Conference of the Systematic Association held in Cardiff, Wales a symposium entitled "The New Taxonomy," and published Special Volume Series 76 (Wheeler 2008, ed.). Participants argued for the important new role of taxonomy in the understanding and conservation of biodiversity. Attention was focused on the inadequacy of funding for taxonomic work and education; the need to support descriptive taxonomy, which is the basis of identification and phylogeny but has recently been the neglected Cinderella of taxonomy; and the urgency of e-taxonomy, networks, museum engagement, democratization of taxonomy and planetary scale projects. Taxon knowledge communities, "people with similar interests and expertise connected through cyberinfrastructure such that they may interact, collaborate and even compete with one another on a much accelerated time scale," (*ibid*.) have taken on important significance. Taxon knowledge communities lead to virtual taxon knowledge banks in which the following are available in a cost-effective and environmentally-friendly manner: literature, 3-D digitized and georeferenced specimens, identification tools, maps, field guides and all of the pertinent information from all taxonomic sources. At the present time, the international scientific community is enabling a New Taxonomy through large-scale projects and programs such as: Encyclopedia of Life (EOL), Global Biodiversity Information Facility (GBIF), Planetary Biodiversity Inventories (PBIs), Legacy Infrastructure Network for Natural Environments (LINNE), European Distributed Institute for Taxonomy (EDIT) and Creating an e-Taxonomy (CATE). The book "The New Taxonomy" is loaded with energy and vision and presents a call to arms "for the taxonomy and museum communities to come together and to organize, plan, innovate and initiate the most ambitious period of exploration in the long history of taxonomy" (*ibid*.).

Ways forward

The scleractinian taxonomy is still far from realizing the progress and advantages of contemporary taxonomic science. How this can be efficiently addressed? Taxonomic work can be oriented top-down or down-up. Both ways are useful. The latter is practiced more frequently and is more matched to everyday necessities. Teamwork can be organized on various different scales: planetary, regional or special task forces. The volume of work and the nature of species composition makes it recommendable to start regionally.

Toward an Atlantic Scleractinia Initiative

The following must be addressed for the success of a future integrative approach to Atlantic scleractinian taxonomy:

- *Massive sampling.* Comprehension of scleractinian variability and plasticity requires massive sampling, which lately is hardly ever applied.
- *Study of the mesophotic habitat*. Despite the well-established scleractinian presence in the mesophotic habitat in Jamaica (Goreau and Wells 1967), Cuba (Zlatarski 1982), Bahamas (Reed 1985), Barbados (Macintyre et al. 1991), Curacao and Bonaire (Bak et al. 2005) and Mexico (Zlatarski 2008), recent attention has mainly been focused on shallow waters.
- *Collection access*. There is much to be done. The collection of T. F. Goreau has for more than three decades remained unavailable in storage.

Among the large monograph collections, only the Cuban one is well-preserved in the National Aquarium in Havana (Zlatarski 2004), but unfortunately, for reasons beyond the control of the host institution, it is out of reach for many researchers. The corals at the Smithsonian Institution, Washington, D.C., serve as a good example of well-inventoried and available specimens. For the Atlantic province, digitized collections and virtual usage of types and specimens are not yet available.

- *Analysis of phenotypic diversity*. Knowledge of phenotypic diversity at all levels of biological organization, from structural elements to high systematic categories, is basic for taxonomic work. The phenoid delineation is a first step for making sense of taxon structure. Its usefulness became evident when taxonomic problems identified and decisions made decades ago (Zlatarski 1982) were recently validated by molecular studies (Zlatarski 2008).
- *Life history*. The insufficiency of data on the life history of Atlantic scleractinians presents an obstacle for a better understanding of the species as a basic taxonomic unit. Systematized observations and experiments in natural habitat and aquaria are needed.
- *Geological history*. The application of the reversed principle of uniformitarianism can facilitate the explanation of particular cases of evolution. For example, the presence of considerably smaller and more delicate scleractinian colonies in Golfo de Guacanayabo, Cuba, as compared to the rest of Caribbean (Zlatarski, 1982), may be viewed together with the endemic morphogenesis observed in Lower Cretaceous scleractinians of Diplocteniopsidae in Bulgaria (Zlatarski 1968). It is likely that evolution in an environment of calm waters, the lack of a hard bottom and the presence of very fine sediments resulted in a different colony astogeny in a very limited temporal and spatial range.
- *Molecular genetics*. As a new, very powerful and expensive taxonomic tool, molecular genetics should be used after the completion of a systematized sampling process corresponding to phenotypic diversity and life history traits, in coordination with interested researchers.
- *Cyberinfrastructure*. A website for Atlantic scleractinian taxonomy is needed to form a taxon knowledge community and taxon knowledge bank, and to provide easy and inexpensive access to literature, collections and all pertinent documentation for specialists and others interested in scleractinians and reefs. The experience of writing the Treatise on Invertebrate Paleontology for Scleractinia by means of a website could be helpful in designing a website for extant Atlantic scleractinians.
- *Education of specialists*. The necessity to make use of four sources of taxonomic information requires fundamental changes in educational programs in order to prepare specialists to able to meet the more stringent requirements of the New Taxonomy.

Ultimately, all of the foregoing recommendations will be fruitless in the absence of a collaborative spirit in forging strategies and working together in the most efficient and integrative way. This is especially important at a time when taxonomy plays such a crucial role for the understanding of scleractinian biodiversity and the conservation of coral reefs.

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Species richness and morphological diversity of the Genus *Porites* in the Pacific Ocean

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Abstract. Species richness is a common measure of biodiversity, but offers only a partial view of the assemblages. Morphological diversity is another indicator, and can reflect functional aspects of the system more efficiently than the number of taxa. In this study we compared patterns of richness and morphodiversity in the genus *Porites* in the Pacific Ocean. Distribution data of 52 species were arranged in quadrants of 20° of latitude and longitude, and nine morphological characters were measured for each species. The latter data were processed with a principal components analysis, and the diversity of forms was estimated from the geometric mean of the range of the scores of each component. We found out that richness in this genus is concentrated in southeastern Asia, but morphodiversity is more widely distributed and high values appear indistinctly if species number is high or low. The relationship between richness and morphology in *Porites* followed a logarithmic curve and tend to reach an asymptote, and there were no significant differences in morphodiversity of the hemispheres or between the tropics and subtropics. We suggest that the observed morphospace limitations in *Porites* may indicate saturation in niche availability, or occur because of the existence of phyletic boundaries.

Key words: coral, Porites, Pacific Ocean, morphology

Introduction

Biological diversity is a complex property that can be measured using any of many traits, from genetic diversity to landscape variety, as each of them offers a different perspective of the phenomenon (Wilson 1996). The most common organization level at which biodiversity has been quantified is the community, and in this case researchers usually focus on species richness as the concept is easy to grasp by the general public and it is intuitively "natural" (Gaston and Spicer 2004). Studies performed in the last decade show that richness tend to correlate well with other biodiversity predictors, and even between different taxonomic groups (Roberts et al. 2002; Qian and Ricklefs 2008). However, in some cases the richness behaves differently to specific and important indicators; for example is not much related to endemism both in the land and marine realms (Hughes et al. 2002; Orme et al. 2005), and because of that situation some authors have called attention to avoid focusing all efforts to preserve only rich areas (Kareiva and Marvier 2003)

One predictor that seems to correlate well with marine species richness is morphological diversity (Roy et al. 2000). The latter has traditionally been considered as a key tool for paleoecological reconstruction (Foote 1992), and other studies have demonstrated its application for the better understanding of the ecological functioning in Recent coral reefs (Bellwood et al. 2006), gastropod evolution (Latioalis et al. 2006), and comparison of niche space utilized by gastropods living at different depths (McClain et al. 2004). The good fit between form and function was well received because in turn, morphology can reflect the functional aspects of the marine ecosystems very efficiently (Wainwright 2007) as it recognizes particular traits of each species; thus, species richness could be applied as an efficient proximal indicator of ecological functions performed by a certain taxonomic group.

However, not all papers coincide that richness and morphology follow a common trend. For example, Foote (1993) found no relationship or clear trend between richness and morphological diversity in trilobites, and in recent marine organisms the number of species of strombid snails turns out to be a poor indicator of morphological diversity in the Indo Pacific (Roy et al. 2001), basically because certain areas with low richness had a high morphological diversity. Other examples of this situation are cuttlefishes, in which richness and morphological diversity and disparity are independent (Neige 2003).

Corals have a long history of morphological analyses, especially related to taxonomy and phylogeny (Powers and Rohlf 1972; Hoeksema, 1989; Weil et al. 1994), but recently the focus has shifted to more functional aspects related to metabolism, light gathering and others (Enríquez et al. 2005; Todd 2008; Hogenboom et al. 2008). Considering this change of perspective, the objective of this work was to compare the patterns of species richness and morphological diversity in the coral genus *Porites,* as a way to provide a different view of the biodiversity of the taxon. This genus has 60 species (Carpenter et al., 2008), a worldwide distribution and is one of the most conspicuous in reefs. It is also recognized by its ecological role as reef builder and habitat to many species of invertebrates and fishes (Veron 2000).

Methods

To determine species richness of *Porites*, we used the information of 52 species appeared in Veron (2000), and updated it with records from more recent papers. The data (presence-absence) were arranged in quadrants of 20° of latitude and longitude, which covered the entire Pacific Ocean (N=48), and a distribution matrix was completed from where richness was calculated by simple sum.

The morphological study involved nine characters that were measured in museum specimens or from photographs: maximum and minimum corallite (taken as an ellipse), number of septa, number of pali, depth of calices, width of corallite wall, size of columella, ration between depth of calice, and maximum diameter, and type of colony (massive, encrusting or ramose). All measures were taken with a precision of $\pm$ 1 mm, and in a minimum of five corallites or images. The set of measurements was standardized (mean= 0, SD = 1) and processed with a principal component analysis, using a covariance matrix (Bakus 2007). This technique allowed the construction of a multidimensional morphospace (Fig. 1) from where we used the geometric mean of the range of the scores of each component to measure the extent of morphological space occupied (Roy et al., 2000, McClain et al., 2004). The last calculation was performed on the data of present species in each quadrant. Finally, a one way ANOVA test (Zar 1999) was applied to look for differences in morphological diversity between hemispheres (north and south), and latitudes (0° to 20°, and 21° to 40°, independent of the hemisphere)

Results

The Fig. 1 shows the empirical morphospace (axis I and II) calculated for the 52 *Porites* species, and we present sketches of the general morphology at each side of the plot. In specific, the left upper corner has species with a platy morphology (e.g., *Porites tuberculosa* Veron, 2000, *P. densa* Vaughan, 1918), at the upper right we found the ramose species (*P. rugosa* Fenner and Veron, 2000*, P. flavus* Veron 2000), and at the bottom those with encrusting and columnar colonies like *P. solida* Forskaal 1775 and *P. heronensis* Veron, 1985, respectively.

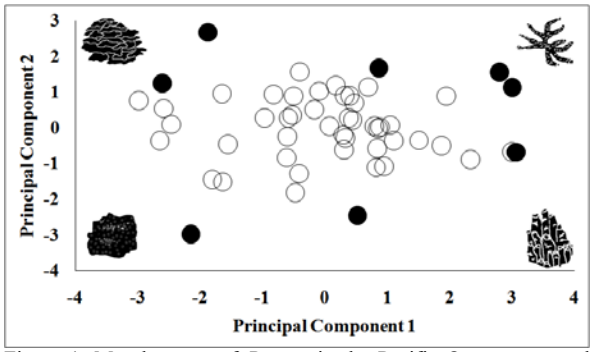

Figure 1. Morphospace of *Porites* in the Pacific Ocean, prepared with principal component analysis.

The map of species richness (Fig. 2) shows that as expected, the highest values can be found at southeastern Asia (over 26 species, and as much as 31), followed by Japan, the Indian Ocean and the Great Barrier Reef (21 to 25). In contrast, the lowest richness occurs in South America, Easter Island and other eastern Polynesian islands, and near the entrance of the Persian Gulf. As it occurs with many marine species, and in particular with corals, there is a clear gradient from the center of marine diversity around Indonesia and Papua New Guinea, to the central and eastern Pacific (Bellwood and Hughes 2001; Roberts et al. 2002).

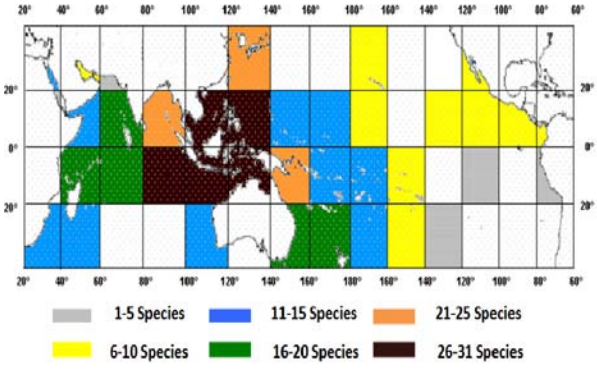

Figure 2. Species Richness of genus *Porites* in the Pacific Ocean.

The situation of morphological diversity was very different (Fig. 3). The index had its highest values in areas including the east coast of Africa, the central Pacific and the equatorial Indian Ocean. It was also

high at the entire north and east coast of Australia and New Caledonia. Interestingly, the areas with lowest morphodiversity (South America and east Polynesia) coincide much more with the results in Fig. 2

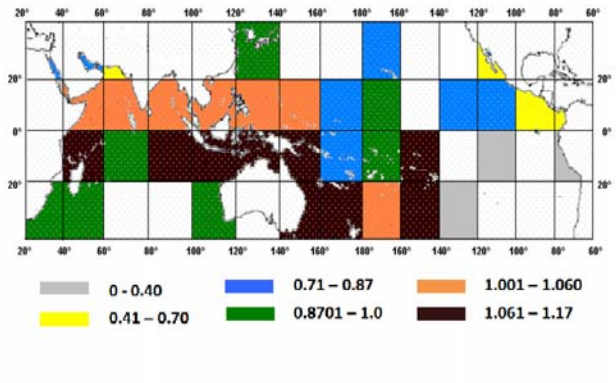

Figure 3. Morphological diversity of the genus *Porites* in the Pacific Ocean.

Comparing species richness and morphological diversity, we can observe that the relationship is not linear, but follows a logarithmic curve. When richness is low 0 to 10 species), the complexity of forms in each quadrat of 20 square degrees rises fast, but in areas where richness is higher than that, the morphodiversity tends to flatten. Also notice that the site where the variety of forms is highest (1.089; eastern Polynesia), there are only seven species present.

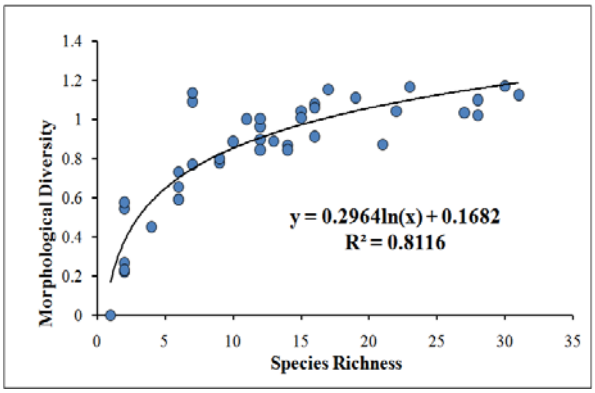

Figure 4. Species richness and morphological diversity in *Porites*. The regression was significant.

To test if sites where richness was higher than 10 can have an effect on morphology, we run a Student t test to compare areas with 10 to 20 species with those having 21 or more. and the result indicated no significant differences ($t_{25} = 1.15$, $p > 0.10$). In short, our analyses indicates that morphological diversity in the genus *Porites* do not increases in proportion to species richness, and that it may exist an upper limit to this biodiversity measure in the Pacific Ocean.

The comparisons between hemispheres (Fig. 4) indicated that richness of *Porites* per site was higher in the Southern Hemisphere than in the Northern ($t_{25} = 3.21$, $p < 0.01$), but that morphodiversity did not differ ($t_{25} = 2.07$, $p > 0.05$). In the case of latitude (Fig. 5), the number of species per site also differed as areas north of 20° N and S were richer ($t_{25} = 5.43$, $p < 0.001$), but as it happened previously, the morphodivesity did not differ between tropical or subtropical zones ($t_{25} = 1.21$, $p > 0.05$).

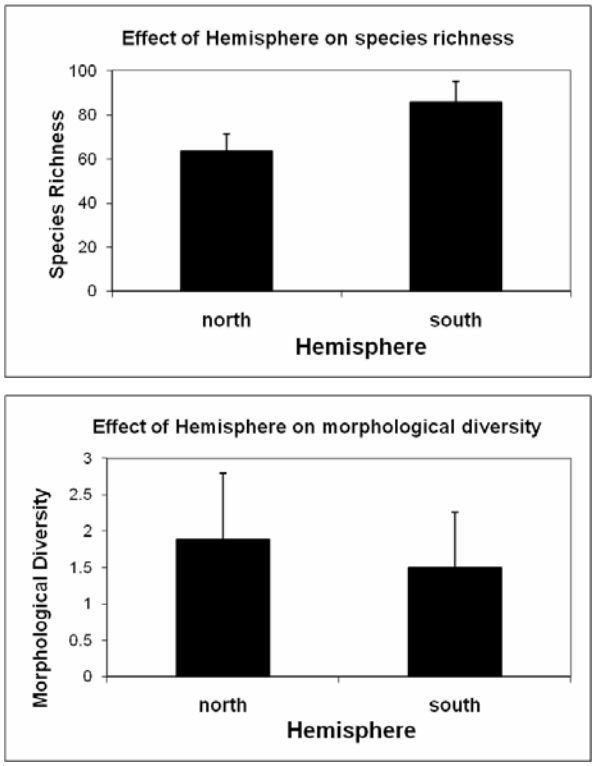

Figure 4. Comparison of richness and morphodiversity of *Porites* between hemispheres in the Pacific Ocean.

Discussion

As shown, morphological diversity and species richness of *Porites* corals behave independently. The scenario that richness generate a variety of forms (and consequently that the relation should be monotonic, or at least positive) has been tested and found in studies of terrestrial animals (Williams and Humphries 1996; Roy and Foote 1997) but nevertheless it does not seem to be the dominant pattern in the marine realm, as it was not found either in gastropods (Roy et al. 2002), sea urchins (González-Azcárraga 2008) or corals (this study). This finding is not necessarily bad news; on the contrary, we propose that the fact that richness and morphodiversity are complementary opens a new

avenue of research in the future, with important ramifications for conservation and functional studies in reef ecosystems.

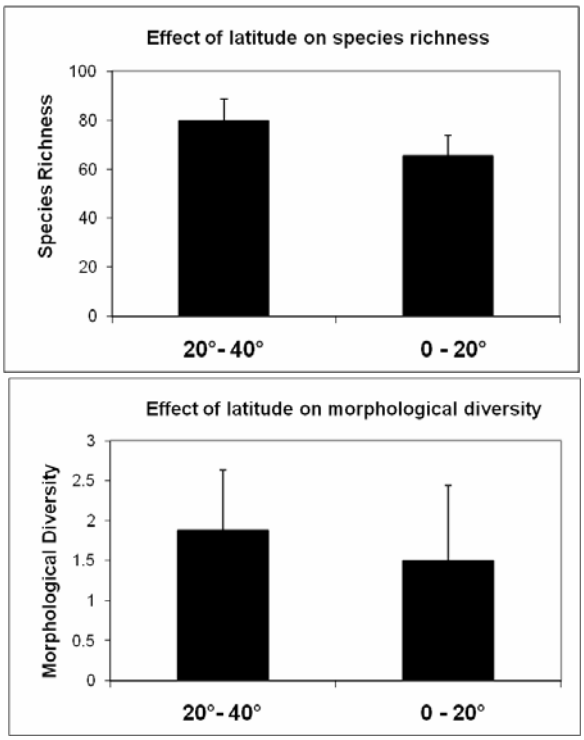

Figure 5. Comparison of richness and morphodiversity of *Porites* between hemispheres in the Pacific Ocean.

In Fig. 3 it is apparent that some regions with few species have remarkably high morphological diversity, including peripheral areas like Polynesia and east Africa. A more in-depth examination should be done, but it is possible that these anomalous areas have at least one endemic species, or a coral with an unusual collection of characters, so the value of the variance in form is increased. However if we consider the Pacific as a unit, morphodiversity in *Porites* is remarkably homogeneous and it seems not to be much influenced by latitude or hemisphere (Figs. 4-5).

Maybe the most important result of the study was the comparison of richness and morphology, which point out that even when both indicators move almost linearly near the axis, the latter eventually tend to reach an asymptote (Fig. 4). This pattern can be explained either from the evolutionary or ecological perspective. In the first one, the genus *Porites* might actually have a phyletic limit in its variety of forms and design (its bauplan), and thus it should be independent of richness. If this idea is correct, a study on fossil species may found that the genus has been transforming in time, and becoming more complex in its calicular and colonial structure. On the other hand, the limitation in morphology may result if the environment is providing only a limited kind or amount of resources, so that eventually result in saturation of niche availability. There have been many discussions on the size of the niche of hermatypic corals, and of the factors that determine it (Connell 1978; Knowlton and Jackson 1994; Iglesias-Prieto et al. 2004), but the authors coincide that as scleractinians are specialists, its ecological space should be restricted. This observation could explain our results, but there still are many questions to be addressed before obtaining a firm conclusion. Whatever the case, the use of morphological diversity as an indicator of biodiversity of reef corals show promise and should be pursued in future research.

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Coral assemblages of Cabo Verde: preliminary assessment and description

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Abstract. A preliminary ecological assessment of coral communities was conducted in Cabo Verde (North Atlantic). Two fieldtrips recorded underwater transects with DAFOR semi-quantitative scale to estimate relative abundance of coral species. This effort, together with a review of historical records, allowed a preliminary identification and description of three major coral biotopes: i) antipatharian facies in vertical walls; ii) *Siderastrea radians* pavements over shallow bedrock; and iii) diverse coral coverage over rocky reef with sand patches. Coral species diversity in these biotopes is lower than in other tropical reef ecosystems. Sal Island, where a more extensive survey was possible, revealed greater living coverage and biodiversity on the leeward side when compared with the windward side of the island. Depth and hard substratum orientation (vertical/horizontal) appear to act as major environmental factors structuring communities in these biotopes. The importance of other factors affecting their distribution, structure and diversity, such as water temperature, West African upwelling influence and dust deposition from Sahara desert, by prevailing and Harmattan winds, are briefly discussed.

Key words: Ecological assessment, coral diversity, biotopes

Introduction

Sited at 15.02N, 23.34W, the Cabo Verde archipelago comprises ten islands and five islets located about 300 nautical miles WNW off the coast of Dakar, Senegal (Fig. 1). Volcanic in origin, the islands rise from the deep abyssal plain beyond the African continental shelf.

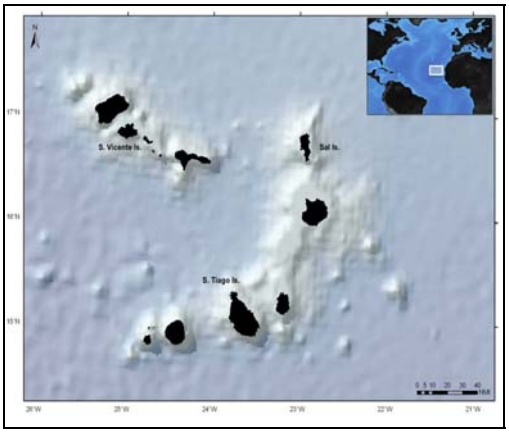

Figure 1: Geographical location of Cabo Verde Islands.©*Imag*DOP

These islands are considered to be an important hotspot of tropical reef biodiversity and one of the top ten priority locations worldwide for the conservation of reef habitats (Roberts et al. 2002). Despite the absence of major coral reef structures and of the relatively low richness of coral species and reef-associated fauna, these islands' coral communities constitute unique habitats as many species display a small distributional range (Laborel 1974; Moses et al. 2003; Roberts et al. 2002).

These coral communities are of major importance for coastal ecosystems (providing habitat and supporting other fauna and flora) and play a key role on the sustainability of local fishing and economy (Wells 1988; Wilkinson 2004).

Recent efforts to characterize coral communities and associated fauna and flora have been conducted in specific locations of São Vicente Island (Delgado 2006) and Sal Island (Henriquez et al. 2006). Although providing valuable information and extensive lists of coral species and associated fauna and flora, studies are site restricted and lack a uniform approach that relates species composition and its variance to environmental conditions.

The goal of the present work was a preliminary ecological assessment of coral communities in Cape Verde Islands, setting the basis for the experimental design of a more detailed characterization of these coral communities. Determining the species composition and its variance within and between locations, related to physical and environmental parameters and gradients, such as depth and substratum nature and orientation, allows one to determine areas of uniform environmental conditions providing a living place for a specific

assemblage of organisms (Jokiel 2002; Wallenstein and Neto 2006). Accordingly, accessing i) conspicuous coral species, relative abundance and distribution and closely associated species composition, and; ii) major physical and environmental parameters/gradients determining coral distribution, provided valuable data and information to identify major coral communities' biotopes and produce the necessary background for following characterization of these communities.

Material and Methods

Fieldwork conducted in Cabo Verde included two survey missions (November and July 2007) at three islands (Fig. 2) where coral communities had been reported by Laborel (1974) and Moses et al. (2003). Within each location, five sites were selected and surveyed using depth and substratum nature and orientation (horizontal/vertical) as major determining factors conditioning coral and associated species' composition and distribution limits.

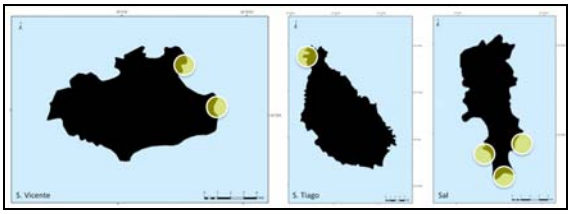

Figure 2: Visited locations during survey missions to S. Vicente Is. and S. Tiago Is. (July 2007) and Sal Is. (November 2007)©*Imag*DOP

Each site was surveyed (using SCUBA) with four underwater transects, parallel to a depth gradient between 5 and 20 meters of depth. Transects were randomly distributed using a two digit random number table. The first digit determined the direction of following placement: heading towards the shore, an even first digits meant the following transect would be placed to the right and odd digits meant it would be placed to the left of the previous transect (first transect placed in relation to the start point of the dive). The second digit determined the distance, in meters, for the placement of the following transect (first transect placed in relation to the start point of the dive). For example (Fig. 3), having the first transect ended at 20 m, the random number 27 would mean that the following transect would start at that depth, 7 meters to the right (heading shoreward). Whenever transect placement would fall outside hard bottom substratum with coral coverage, the following random number would be considered.

Coral species relative abundance was accessed with DAFOR (**D**ominant, **A**bundant, **F**requent, **O**ccasional and **R**are) semi-quantitative scale (Hawkins and Jones 1992, Wallenstein and Neto 2006), at each 5 meters of depth. Conspicuous associated fauna and flora composition substratum nature and orientation was also accessed throughout these transects. Hard bottom substratum, where coral occurs, was classified into three categories: i) bedrock, ii) bedrock patches and boulders over sand bottom, and iii) vertical and sub-vertical walls. DAFOR values were averaged from transects over the three major kinds of substratum where coral species occur for overall values.

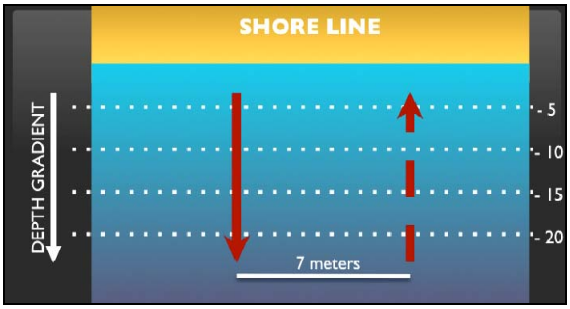

Figure 3: Scheme of two consecutive transects' placement using a random number equal to 27. Second transect (dashed) start point placed 7 meters to the right of previous transect end point.

Results

Biological data against depth, bottom type and orientation suggested that these physical parameters determined local coral species composition and limits. Restricted to hard bottom substratum, coral species distribution and DAFOR values were fit into the three categories of hard bottom substratum. Raw transect data were averaged within each category and depth (Table I). These values refer exclusively to coral species, describing relative abundance within this group.

Based on depth gradient, bottom type, orientation, as well as coral species composition, distribution and relative abundance, three different biotopes were identified:

Shallow Rocky Reef

Rocky platforms, reefs and bedrock between -4 m and -10m were clearly dominated by *Palythoa caribaeorum*, which overgrew several other coral species such as *Porites astreoides* and *Siderastrea radians. Millepora spp, Porites spp.* and *Siderastrea spp.*. *P. caribaeorum* dominance was clearest at Sal Is. and less so at other islands, where the species was dominant or abundant but did not overgrow other corals.

Siderastrea Pavements

Sandy bottom substratum, with bedrock patches and medium boulders, between -5 m and -15 m (occasionally to -20m), restricted to sheltered bays. A clear *Siderastrea spp*. dominance, forming pavements over almost all available hard substratum.

Table 1: Coral species relative abundance over different substrata (overall DAFOR values, D=Dominant, A=Abundant, F=Frequent, O=Occasional and R=Rare). * On the bottom edges over sand.

	Bedrock Reef				Bedrock patches and boulders				Vertical wall			
	5 m	10 m	15 m	20 m	5 m	10 m	15 m	20 m	5 m	10 m	15 m	20 m
ANTIPATHARIA												
Antipathella spp.	-	-	-	-	-	-	-	-	-	O	D	D
Stichopathes sp.	-	-	-	-	-	-	-	-	-	R	F	O
Tanacetipathes wirtzi	-	-	-	-	-	-	-	-	-	O	A	A
GORGONACEA												
Leptogorgia capverdensis	-	-	-	R*	-	-	R*	F*	-	-	R*	F*
Leptogorgia spp.	-	-	-	R*	-	-	R*	F*	-	-	-	F*
SCLERACTINIA												
Favia fragum	O	O	-	-	O	O	O	-	-	-	-	-
Madracis sp.	-	-	-	-	-	R	-	-	-	-	R	-
Porites astreoides	A	A	O	-	O	O	O	-	-	-	-	-
Porites sp.	F	-	-	-	O	R	-	-	-	-	-	-
Siderastrea radians	F	F	-	-	D	D	D	D	-	-	-	-
Siderastrea sp.	O	R	-	-	A	A	F	F	-	-	-	-
Schizoculina africana	-	-	-	-	-	-	-	R	-	-	-	R
Tubastrea spp.	R	-	-	-	-	.	-	-	-	D	A	O
Dendrophyllia spp	R	-	-	-	-	-	-	-	-	R	-	-
ZOANTHIDEA												
Palythoa caribaeorum	D	D	-	-	-	O	-	-	-	-	-	-
MILLEPORINA												
Millepora spp.	A	O	-	-	O	F	R	-	R	-	-	-

Occasional presence of *Porites spp., Favia fragum,* and *Schizoculina africana*. Presence of *Leptogorgia spp,* over sand at the edges of hard substratum.

Walls

Vertical and subvertical facies between -10 and -20 m were dominated by antipatharians with low or no presence of scleractinians other than *Tubastrea spp.* that were abundant at lower depths, in crevices and other places with low lighting. *Leptogorgia spp.* was common over sandy bottom close to the lower edge of these walls.

Associated Fauna and Flora

Qualitative assessment of associated fauna and flora showed a clear dominance by *Dictyota spp.* and a strong presence of *Asparagopsis taxiformis* over horizontal hard bottoms. *Caulerpa sp.*, *Codium sp.* and cyanophytes were similarly abundant over bedrock patches with sand deposition. Conspicuous fauna within these biotopes include the sponges *Aplysina aerophoba* and *Chondrosia reniformes*, the gastropod *Stromba sp.,* nudibranchs *Hypseledoris spp.* and the polychaete *Hermodice carunculata.* Ichthyofauna over horizontal substrata included *Myripristis jacobus*, *Coris julis*, *Chromis multilineatus*, *Synodus saurus*, *Sparisoma cretense.* On vertical walls *Myripristis jacobus, Aulostomus strigosus, Sargocentron hastatum* and *Gymnothorax miliaris* were the most abundant species.

Discussion

This preliminary ecological assessment reveals importance of depth, type of substratum and orientation on community structure (Veron 2000; Wells 1988; Wilkinson 2004). Surveys at Sal Is. suggest a difference between living coverage on the leeward and the windward sides of the island. Factors such as temperature might be acting on a local scale due to prevailing currents. Wind regime, West African upwelling and currents together with cyclic Harmattan winds (blowing offshore from the African mainland) may cause significant deposition of dust on the windward (East) side of the island, with an impact on turbidity and light penetration in the water (Moses et al. 2003; Shinn et al. 2000) besides the negative effect of sedimentation on the organisms. The dominance and overgrowth of *P. caribaeorum* was striking at some sites. This species is known for its resilience and for smothering other corals by overgroth (Suchanek 1981). It may thus be a potential hazard for local coral communities and should be monitored.

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